ANNUAL REPORT
OF
PROGRAM ACTIVITIES

DIVISION OF BIOLOGICS STANDARDS

FISCAL YEAR 1972

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE NATIONAL INSTITUTES OF HEALTH

ANNUAL REPORT

OF

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U.S. DIVISION OF BIOLOGICS STANDARDS

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DIVISION OF BIOLOGICS STANDARDS

ANNUAL REPORT

July 1, 1971 through June 30, 1972

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DIVISION OF BIOLOGICS STANDARDS

SUMMARY OF THE DIRECTOR

INTRODUCTION

The mission of the Division is the exercise of controls on the safety, purity and potency of biological products through a system of licensing of manufacturing establishments and of the products, as well as the conduct of research related to these responsibilities in order that decisions made and actions taken can be based on sound information.

The Division has the statutory task of making recommendations for standards for the continued safety, purity and potency for all biological products such as vaccines, serums, human blood and its derivatives, and like products. These include written standards, eventually promulgated as regulations, some of which apply to individual products and some to products in general, and physical standards, that is, preparations whose properties and qualities have been extensively characterized and which are issued to manufacturers and other research laboratories concerned with the standardization of biological products for evaluation with their own products, particularly with respect to potency or strength.

The acquisition of the information needed to develop written standards and the laboratory investigations leading to the development and selection of suitable physical standards are extensive in character and often time-consuming. This is particularly true of standards for the tissue-culture-prepared virus vaccines such as poliovirus, measles, mumps and rubella, which have been developed in recent years.

When a new vaccine against a particular disease appears achievable, it devolves upon the Division to develop standards that will be ready at approximately the time that the product is brought to the final stages of manufacture. This requires considerable research involving most of the component laboratories of the Division, as well as the testing of samples of both experimental and final products. It is necessary during this period to maintain close contact with the manufacturer and with scientists working in the field so that problems related to standards can be resolved during the developmental phases. In addition, any clinical investigations conducted by the manufacturers or by investigators studying the products under a variety of conditions come under review of the Division.

When a manufacturer decides to market a product which he has developed, he submits a license application to the Division, together with samples of the product and results of all tests. Division scientists then review the application and examine the product in the laboratory to ascertain the validity of the manufacturer's test results. If the tests fulfill the requirements and it can be shown that applicable standards are met, a product license is recommended for approval. If this is the first product subject to license by the manufacturer, his facilities are also inspected, and if established

standards are met, he is simultaneously recommended for an establishment license. In the case of a manufacturer already licensed for other biological products, production facilities may be inspected as deemed necessary according to the nature of the pending product. The establishment is inspected annually to verify that proper procedures are being followed in manufacturing and testing. Samples and the results of tests performed on each batch or lot of biological products proposed for marketing must be submitted to the Division by the manufacturer prior to distribution, as determined by the Director. The Division checks the test results and runs similar tests to ensure the quality of the products. Only then is a release which permits marketing issued to the manufacturer.

Once a product has come into use, its safety and potency must be monitored. The knowledge and skill required to do this also provides a sound basis of knowledge and experience for consideration of problems associated with newer products as they are developed.

Brief reviews of the control activities, and of licensing inspections, investigations* and legal actions are included in this summary.

Continued research on both old and new products is necessary, not only for obtaining information leading to development of improved standards, but also for the development of expertise and scientific competence on the part of the staff. In many respects, this is a more compelling inducement towards compliance than recourse to regulation-based actions.

Because of the Division's concern with products as such, and because of the involvement of the research staff in the regulatory function, a great deal of attention is directed to "applied" research problems. However, there is probably no element within the NIH where there are more examples of the difficulty of using the words "applied" and "basic" truly to define research endeavor.

The scientific staff continually studies new methods and new approaches to the problems associated with biological products because significance of new knowledge in relation to these products is essential to its function. Of continuing major concern are the viral vaccines, some of which, like vaccinia and rabies, are among the first licensed products, while newer ones are continually being developed. The Division must attempt to evaluate the significance of the demonstrated potential of some viruses to produce tumors in animals under special circumstances; it must also be concerned with the problems of covert viruses which may contaminate cell cultures, and other systems used for the propagation of viruses. This requires that older products be reexamined in terms of contemporary scientific advances from time to time.

The development of new respiratory viral vaccines, and the continual reassessment of older products such as inactivated influenza vaccine, require the Division to develop knowledge concerning antibodies involved in serum in comparison with those in respiratory tract secretions, as well as information on the sites of production and on the transport of these antiviral substances across cell membranes. This is particularly true of inactivated vaccines

and is considered to be responsible for the less than optimal potency of influenza vaccine, for example. Analogous problems exist in regard to the factors possibly related to hypersensitivity which have apparently contributed to severe illnesses resulting from natural infection following artificial immunization with some experimental inactivated respiratory vaccines, produced from such agents as respiratory syncytial virus and *Mycoplasma*. These are also related to cellular aspects of immunity, hypersensitivity, and allergy, all of which are problems attracting the attention of DBS scientists, as well as scientists working in the fields of immunology, microbiology and epidemiology.

The study of blood proteins also extends beyond their relation to infectious diseases because of the Division's concern with blood and blood products. Study continues on the purification of various plasma components involved in the clotting and fibrinolytic systems.

At the same time, the Division is still confronted with such continuing problems as improvement of pertussis and possibly of tuberculosis vaccines.

New techniques of administration of vaccines and other biological products are also studied since these require careful appraisal. In addition, proposals for the use of various oil adjuvants in immunization and in allergy hyposensitization have prompted the Division to develop an extensive series of contract supported research projects to help resolve the issue of the safety of these materials.

The scientific pursuits of the DBS staff represent responses to solve problems related to its mission. To some extent, the staff must initiate investigations because of proposals for new products being developed by other organizations. Similarly, the control functions lead the staff to recognize gaps in existing knowledge of various biological products and their effects. Also DBS scientists, like their colleagues in other organizations, are motivated by adding to the body of knowledge in their fields.

SCOPE OF THE CONTROL-TESTING AND LABELING PROGRAMS

The control-testing program operates as follows: Manufacturers of a licensed product submit to the Division samples from each lot of the product, together with protocols containing the results of tests performed on that lot. Some 227 different biological products are currently licensed involving the issuance of 1,189 individual product licenses.

Each protocol is reviewed by the Division to determine if the manufacturer's test results show conformance to established standards. In addition, some or all of the safety, purity and potency tests required on a particular product may be performed by the Division *as a check on the testing performed by the manufacturer*. If the manufacturer's test results and the results of tests performed by the Division are satisfactory, an official release for that lot is sent to the manufacturer. Only then may the manufacturer distribute the product lot for sale under license.

Two tables are included here to summarize the Division-wide control effort. During the 12-month period covered by this report, 6,105 lots of biological products were released, 45 lots were rejected for a variety of reasons, and 51 lots were voluntarily withdrawn by the manufacturer. Table 1 reflects the number of lots released by categories of products.

A protocol showing the manufacturer's results may consist of considerable information requiring up to 200, and, in many cases more, pages, for example, on a lot of Poliovirus Vaccine, Live, Oral, or data on only a page or so for a lot of blood grouping serum. All protocols are recorded and reviewed by members of the Protocol Review and Product Release Section. The protocols are then forwarded to other laboratories within the Division for review, according to their particular expertise. The nature of the product determines the laboratory(s) consulted. For example, a protocol for Measles Virus Vaccine, Live, Attenuated may be reviewed by personnel in many of the Division's laboratories.

Some of the Division personnel are involved in the control program on a full-time basis while many others participate intermittently as needed according to the subject. As the testing of products becomes more specialized, a larger number of the research scientists become engaged in control activities.

Table 2 shows the total number of control tests performed by Division personnel during the last year. This gives no measure of the total effort involved. Tests requiring 15 minutes or less are given the same weight as those taking a month or more to complete in this type of compilation. Continual attention is given to test development, and progress in these endeavors is to be found in the project reports of the different laboratories. It is in this area, especially, that staff of the Laboratory of Control Activities work in collaboration with other Division scientists.

The control activities of the Division also require the maintenance of a large number of different standards and reference materials. These are prepared within the Division or obtained by contract. They are all evaluated and standardized in the Laboratories of DBS and distributed as needed. During the 12-month period covered by this report, 27,646 individual preparations were issued, a marked increase over the 19,471 preparations issued during FY71 (April 1, 1971 to March 30, 1972).

The Protocol Review and Product Release Section maintains a file of complaints and reactions to biological products as received by the Division. Included in the file are copies of complaint correspondence and information on any follow-up actions as required. Also retained in the office is a record of lots of biological products recalled from distribution and use either by the manufacturer or as the result of a request by the Division.

The Label Review Section, Office of the Director, reviews and coordinates the review of all labeling used for biologic products. Such labeling includes container and package labels and package circulars used in both domestic and foreign commerce. Each label is carefully reviewed to determine whether compliance has been achieved with Sections 73.600 through 73.605 of the PHS Regulations as well as applicable Regulations issued by the Food and Drug Administration.

The majority of the labels reviewed were for blood, blood products and laboratory diagnostic substances. The primary review of these labels was performed in the Laboratory of Blood and Blood Products by personnel with special technical training in this field. Similarly, primary review of labels on certain bacterial and viral products was performed in the appropriate laboratory by personnel possessing specialized training in these respective disciplines. All such labels undergo an additional review in the Label Review Section.

During the period covered by this report, approximately 2,100 pieces of labeling were reviewed and commented upon before being returned to the manufacturer. This represents about a 5% increase over 1970.

TABLE 1

Product Group	Lots Released	Lots Rejected	Lots Withdrawn	Totals
Antitoxins	12	0	0	12
Therapeutic Serums	9	0	0	9
Blood and Blood Products	2271	8	13	2292
Bacterial Vaccines	125	0	3	128
Toxoids and Toxins	53	0	1	54
Multiple Antigen Preparations	155	4	4	163
Viral and Rickettsial Vaccines	1193	9	7	1209
Diagnostic Substances for Dermal Test	132	1	0	133
Diagnostic Substances for Laboratory Tests	1945	22	22	1989
Miscellaneous	210	1	1	212
Totals	6105	45	51	6201

TABLE 2

TOTAL CONTROL TESTS PERFORMED IN REPORTING PERIOD

	Type of Test	Number of Tests
Potency	In Vivo	1,379
	In Vivo	8,570
Safety	General	969
	Specific	12,602
Purity		12,089
Total Control Tests		35,609

SUMMARY

Program direction and administration for the Division of Biologics Standards is based in the Office of the Director, with centralization of administrative procedures relating to personnel, supply and accounting. Major objectives are determined and program responsibilities are assigned to the operating units within the Division. For each major area, staffing patterns are developed and are continually reevaluated in relation to program changes. The required support is determined for each area in relation to the Division's total budget. A major activity of the Director's office is the maintenance of the proper balance between control activities and research programs.

The Public Information Office is primarily responsible for: 1) Communicating to the public thru the media (press, radio, and TV) the significant aspects of the Division's control and research activities; 2) Handling telephone calls and mail inquiries concerning biologic products related to the prevention and cure of diseases in man, from government agencies, university and industrial research laboratories, physicians, pharmacists, teachers, students, and the general public (material relating to the Division's programs--publications, reprints, brochures, fact sheets, and backgrounders--are utilized in answering these requests); and 3) Providing internal reports on request to the NIH, DHEW, and Congress.

The tasks of the Biometrics Section represent the performance of work, using already established statistical techniques, for solving problems related to testing of biological products originated in various laboratories. In addition, the Section is involved in studies directed toward perfecting statistical methods used in bioassays and to serving the research needs of the Division. It provides biostatistical service for the entire Division.

The Investigational New Drugs Office receives and examines "Notices of Claimed Investigational Exemption for a New Drug," which are required by Section 130.3 of the Food and Drug Regulations. It functions within the Office of the Director. During the period covered by this report, 80 "Notices" were received. This brings to 514 the total number of "Notices" received since the Food and Drug Regulations requiring such submissions became effective. The Office has carried on an extensive correspondence to correct deficiencies in submission, to point our possible hazards and to improve investigation protocols. The "Notices" add to the Division's alertness regarding the development of new products. It provides the staff with the opportunity to "tool up" in advance for the formulation of regulations and standards for such new products through this mechanism.

The Office of Scientific Communications is responsible for establishing objectives and program policy to meet the scientific and technical information needs of the control and research programs of the Division. It carries out a scientist-to-scientist information program that provides advice, assistance and documentation on the vital scientific decision-making issues.

PERSONNEL ORGANIZATIONAL CHANGES

Numerous organizational changes to improve the effectiveness of the Division took place during this reporting period.

Three laboratories were reorganized.

The Laboratory of Viral Immunology added two new sections. Dr. Lewellys F. Barker was appointed Chief of the new Viral Biology Section and Dr. J. Anthony Morris was appointed Chief of the new Slow, Latent and Temporate Viruses Section. In addition, Mrs. Hope E. Hopps was appointed Chief of the Immunology Section after acting for many years as the assistant chief.

The Laboratory of Blood and Blood Products also added two new sections. Dr. Pinya Cohen was appointed Chief of the new Plasma Derivatives Section and Dr. J.N. Ashworth will be Acting Chief of the new Immunology Section. The name of the Blood and Blood Derivatives Section was changed to the Blood Bank Products Section with Miss Madge Crouch appointed Chief. Mr. Clarance Szwed was appointed Chief of the Diagnostic Reagents Section.

The Laboratory of Pathology was reorganized in April 1972 with the following changes. Dr. Stanley H. Singer was appointed Assistant Chief of the Laboratory. The title of the Histopathology Section was renamed the Experimental Cytology Section with Dr. John C. Petricciani as Chief. The title of the Animal Test Section was changed to Primate Biology Section with Dr. Douglas Lorenz as Acing Chief.

Dr. Ruth L. Kirschstein was appointed Assistant Director (Research Contracts) February 9, 1972. She also retains her functions as Chief of the Laboratory of Pathology.

The following personnel changes also took place. There were four transfers -- two in and two out of the Divison. Dr. Carl E. Miller transferred to NCI and Dr. John C. Feeley accepted a position with CDC in Atlanta. Dr. Robert S. Northrup transferred from NIAID to the Laboratory of Bacterial Products and Mr. Carl F. Piepoli joined the Laboratory of Control Activities from Ft. Detrick.

Harry T. Aylor retired after 36 years of government service.

The Division received 7 commissioned officers in July 1971 and will lose 6 in June 1972.

Dr. Daniel E. Thor, received a medical retirement from the commissioned corps in August 1971, and has accepted the position of Associate Professor of Microbiology at the University of Texas Medical School at San Antonio.

LICENSING

Licensing actions are based on the recommendations of license review committees appointed by the Director from the scientific staff concerned with the particular kind of product or establishment.

As of March 31, 1972, 237 establishment licenses were in effect, together with 111 subsidiary establishments and 264 blood bank donor centers. This represents an increase of 1 establishment license since April 1, 1971, the date taken for the last report. This difference is due to the issuance of 15 new licenses and the revocation without prejudice of 14 licenses.

During the 12 months covered in this report, 86 product licenses were issued, 143 were revoked without prejudice at the request of the manufacturers and none were revoked for cause. As of April 1, 1972, 1,189 product licenses were in effect for 227 licensed products.

During this period, all existing licenses for the following products were revoked at the request of the manufacturers:

Poliomyelitis Immune Globulin (Human)
Anti-rh^{w} and Anti-K Serum (Anti-(C^{w} + Kell))
Antihemophilic Globulin (Human)
Pheumococcus Typing Serum
Anti-Gr(V^{w}) Serum
Anti-Kp^{b} and Anti-K Serum (Anti-Rautenberg and Anti-Kell)
Anti-Rh_{o} + *Rh_{o}* (Anti-D + D^{u})
Anti-Wr^{a} Serum (Anti-Wright)
V. *Septique* Antitoxin
Measles Virus Vaccine, Inactivated
Bacterial Vaccines made from:
- Acne Diplococcus
- Brucella Abortus
- Brucella Melitensis
- Dysentery Bacillus
- Meningococcus

Bacterial Antigens made from:
- Colon Bacillus
- Gonococcus

Modified Bacterial Antigens made from:
- Gonococcus
- Paratyphoid Bacillus A
- Paratyphoid Bacillus B
- Typhoid Bacillus

Also, 75 establishment amendments and 151 product amendments were approved during this 12 month period. At the time of this report, there were 29 applications and 21 amendments for establishment licenses and 116 applications and 126 amendments for product licenses pending.

Among the product licenses issued during the year are three which are new products:

Measles, Mumps and Rubella Virus Vaccine, Live
Measles and Rubella Virus Vaccine, Live
Albumin Microspheres (Human).

INSPECTIONS

Forty four members of the scientific staff, selected by the Laboratory Chiefs and the Office of the Director for their special knowledge, are authorized by the Secretary, Department of Health, Education and Welfare to serve as inspectors. These individuals perform inspections as planned and coordinated by the Licensing and Investigations Section of the Office of the Director. Twelve additional professional employees assist in the inspections as trainees.

From April 1, 1971, through March 31, 1972, the following were the number of inspections performed:

	Scheduled	Unscheduled	Total
Trips completed	86	45	131
Establishments inspected	231	41	272
Locations inspected	98	5	103
Donor Centers inspected	107	3	110*
Man Days for inspections	613	109	722

Also visited were 28 unlicensed establishments that supply material to licensed manufacturers under the short supply provisions of Section 73.240 of the PHS Regulations.

*Donor centers are inspected on a rotational basis about every other year.

INVESTIGATIONS

Investigations are conducted by inspectors from the Office of the Director. In contrast to routine inspections of manufacturing establishments, which call for expenditure of predictable periods of time, investigations must necessarily be carried out for as long and indefinite a period as is required to obtain all the needed information on a reported or discovered possible infraction of the law. Investigations must therefore be carried out by people who have this duty as their main function. Investigations usually require extensive follow-up as the need is indicated in the course of assembling complete and accurate evidence. If prosecution is necessary, the Justice Department is provided with specialized subject matter guidance, expert witnesses are located and assistance is given in the organization and preparation of evidence for the most effective presentation. In some situations it is necessary to call upon laboratory resources for assistance, depending either on the product involved or the nature of the alleged violation.

The Investigations Unit's functions frequently are of mutual interest to other Government agencies. For this reason the Unit maintains a continuing liaison with such agencies as the Justice Department, Department of Agriculture, U.S. Customs Bureau, Post Office Department, Federal Trade Commission, and the Food and Drug Administration.

The Investigations Unit is also responsible for acting on reports of hepatitis as they relate to source material used in the manufacture of biological products or to the final product itself. This required numerous interviews and extensive record reviews to obtain the necessary information upon which a decision can be made regarding the safety of the products involved.

From April 1, 1971, to March 31, 1972, investigations involved a total of 46 1/2 man-days off the N.I.H. reservation. This included visiting both licensed and unlicensed establishments for enforcement purposes in addition to interviews and consultations with other federal and state agencies, distributors, witnesses, and others.

Voluntary Compliances

Four signed memoranda of voluntary compliance were obtained during this period. These involved an overseas shipment of partially manufactured plasma to an unlicensed establishment, a shipment of hyperimmune serum in interstate commerce, the shipment of unlicensed Hepatitis Associated Antibody in interstate commerce and the shipment of plasma to a licensed establishment which had not been manufactured according to section 73.240 of the Regultions.

Customs Detentions

There were six detentions of biological products at ports of entry; five from Europe and one from Central America.

Hepatitis Follow-Up

There were two hepatitis follow-ups which involved plasma intended for use in the manufacture of biological products, from donors who subsequently were found to be HAA positive or who developed clinical hepatitis.

Legal Actions

An establishment which manufactured and distributed Hepatitis Associated Antibody (Anti-Australia Antigen) in interstate commerce without a license pleaded guilty to 12 counts. The corporation was sentenced to a $6,000 fine and court costs.

Two cases were presented to General Counsel. One concerned alleged falsification of blood donor records by a licensed establishment and the other concerned the alleged use of a U.S. license number on units of Whole Blood (Human) and Red Blood Cells (Human) by an unlicensed establishment.

CONTRACTS PROGRAM

The contracts program is now in its sixth year of expanded operation. To date, approximately forty-two contracts have been completed and twenty-one are in progress.

During the past year, this program has been coordinated and directed by a newly appointed Assistant Director. The contracts program of the Division is basically an extension of its intramural integrated program of research and control testing. Such a program enables the Division to continue to perform its expanding regulatory and research functions in spite of a limitation of personnel.

Data obtained from these contracts have proven valuable in providing the Division with information concerning the safety, purity and potency of biological products and leading to the development of standards. Developmental studies designed to produce subhuman primate diploid cell strains have continued to be successful and candidate strains of cells are presently being distributed to interested investigators. It appears possible that such cell strains may serve as substrates for viral vaccines in the future. Studies related to a number of possible developmental biological products such as skin test antigen for diagnosis of coccidioidomycosis, to the use of oil adjuvants and concerning the pathogenesis of hepatitis have been very successful.

The contracts are monitored by Project Officers who are investigators from the individual laboratories. The scientific staff of the Division contributes directly to the development and function of the contracts program. This contribution has been of particular value in the initiation, supervision and evaluation of the contract efforts. Often this has involved considerable expenditure of time, made at the sacrifice to the individual's personal research interests.

Details of each contract will be found in "Contract Narratives." (See Table of Contents for Contract Narratives.)

RESEARCH ACTIVITIES

This summary of the programs of the laboratories reflecting the interests of Division scientists will be identified with the laboratory of the principal investigator. There is, however, much inter-laboratory collaboration not reflected here.

SECTION ON EXPERIMENTAL VIROLOGY (OD)

This Section is concerned with certain aspects of viral carcinogenesis, particularly with the circumstances under which neoplastic changes in cells can be enhanced or suppressed.

Methods for recovering simian virus 40 (SV40) from SV40 induced tumors transplanted in adult hamsters is being used in attempts to recover virus from human carcinomas.

Since some of the hanster tumor cells are virus producers, it can be speculated that some human cancer cells may also produce infectious virus.

These studies have shown that repeated injections, of a non-oncogenic variant of adenovirus type 12, protects hamsters against tumor development if the newborn are infected with the oncogenic source strain.

Preliminary experiments suggest that the non-oncogenic strains of adenovirus type 12 are unable to stimulate transplant immunity which suppresses the growth of transplanted tumors. Attempts to develop a non-oncogenic strain of SV40 have not been succwssful.

Attempts to induce or enhance oncogenicity in animals with non-oncogenic adenovirus strains or with herpes-like virus have also been unsuccessful.

This program provides information on immunity in relation to tumor development and contributes to the understanding of possible hazards in biological products under development.

Laboratory of Bacterial Products (LBP)

The program of the Laboratory of Bacterial Products includes a variety of research projects. Some are designed to investigate basic mechanisms of allergy and infectious diseases, while others involve applied research designed to develop new or improved standards and testing procedures for products subject to license under the Public Health Service Act.

Allergenic Extracts: The potency assay for extracts of short ragweed pollen is now a practical assay and is being used to study stability and uniformity of commercial and laboratory preparations of such extracts. In this way it may be possible to assign storage and dating periods for products of this type based on the known properties of the product. There is, however, a serious need for additional assay methods so that potency assays and reference preparations can be prepared without the need for isolation of a pure allergen from each source. New procedures for this purpose based on a radioimmune assay are under investigation. In addition, a bioassay is under development for bee venom, a product that may have wider use in the future.

Poison Ivy and Poison Oak: Assays for the active principles of these products have been developed and it has been demonstrated in collaboration with a contractor that the active principle can, in fact, reduce the sensitivity of sensitive humans. It should now be possible to apply this information to licensed products. Studies in animals are designed to elucidate the basic mechanisms of sensitization and tolerance.

Mycoplasma: New species of mycoplasma continue to be uncovered. M. primatum was isolated from oral and urogenital tissue of the African green monkey. M. conjunctivae was isolated from outbreaks of keratoconjunctivitis of sheep and goats.

Mycoplasma and acholeplasma continue to be serious problems in the manufacture of biological products since they are frequent contaminants of bovine serum used in cell culture. Their presence is a continual problem to manufacturers of products derived from cell cultures. New and improved methods of isolation and identification are being developed to study this problem.

Bacterial Toxins: Tetanus toxin is composed of at least two components, a neurotoxin and a hemolysin, tetanolysin. Studies on the mechanism of action of the hemolysin revealed that it disrupts lysosomes and platelets releasing enzymes and serotonin.

Biologic Products as Interferon Inducers: The potency of certain biologic products has not been subject to measure; these include mixed bacterial vaccines and house dust extract. Studies in mice now demonstrate that some of these products are interferon inducers, and that house dust extract can non-specifically reduce certain viral infection in mice. It thus appears that these products have a biologic potency that may account for the claims made for them in the treatment of human disease. This is only a beginning, but suggests that much useful work needs to be done with products of this type.

Mycobacterial and Mycobacterial Antigens: BCG continues to be an organism of considerable interest. Although all current strains originated from that isolated at the Pasteur Institute, they have been subcultured many times in many parts of the world. Many of these strains differ in their cultural characteristics, but a thorough analysis of the antigens in culture filtrates established a remarkable similarity among all strains.

Much effort has been expended in improving the specificity of the tuberculin reaction. A new analysis of the culture filtrate antigens of M. tuberculosis and a variety of mycobacteria representing the 4 Runyon groups failed to reveal any antigen unique for M. tuberculosis. It reduces further the probability of finding a completely specific tuberculin test.

Cholera: It has now been demonstrated conclusively that cholera toxoid prepared with formaldehyde is unstable and can revert back to toxin. A cholera toxoid prepared by inactivating the toxin with glutaraldehyde is now under study. This product appears to be stable and immunogenic in rabbits and monkeys.

The role of cholera toxin in inducing the diarrhea characteristic of the disease was investigated. Intestinal loops in monkeys, when challenged with toxin, produced diarrhea fluid. Continued challenge rendered the loop resistant, a condition that seemed to correlate with the development of antitoxin.

Studies on El Tor and Classical strains of V. cholerae showed that their relative sensitivity to sulfamethoxazole and trimethoprim was a useful means of differentiating these strains.

Typhoid Vaccine: The importance of Vi-antigen is still not established, but has now been demonstrated to be a protective antigen in mice. To continue studying this antigen, an improved method of isolation was found that results in an antigen at least 10 times more protective than those previously isolated. A new sensitive and specific method for the assay of this antigen has been developed.

Pertussis: Work has been in progress for some years in developing an improved mouse strain for the pertussis potency and toxicity tests. In cooperation with the Division of Research Services, some mouse strains have been developed that give promise of being better than that currently employed.

LABORATORY OF BLOOD AND BLOOD PRODUCTS (LBBP)

The Laboratory of Blood and Blood Products has five principal responsibilities, each of which requires approximately equal attention since all bear directly on regulatory obligations of the Division for this group of products. These responsibilities are: 1) inspections of establishments 2) testing and evaluation of products 3) review of applications for establishment and product licenses 4) preparation of regulatory standards and 5) scientific investigations related to the needs of 1) to 4). The portion of time devoted to research is lower than other Division Laboratories at the present time because of personnel reductions, increases in establishments and products and the preponderance of blood and blood products manufacturers over those of other biological products.

The research program is directed to gaining the scientific information needed to carry out the demands of the regulatory program of the Laboratory. Investigations in each Section relate to the particular products for which that Section is responsible. The following examples illustrate also the wide diversity of products that are made by different manufacturing methods.

The Coagulation Section is studying methods of purification and characterization of the principal components of the clotting system such as Factor VIII (antihemophilic factor), Factor IX (Christmas disease factor) and fibrinogen all of which are licensed products. More knowledge of these and their associated factors will permit improvement in assay methods.

The Plasma Derivatives Section investigates the properties of plasma products such as albumin, immune globulin, plasma protein fraction and radionuclide labeled proteins, all on the market as licensed products. Stability studies involve investigations of the molecular size of each protein when stored various lengths of time at different temperatures. The immunologic characteristics of these molecules after processing and storage are of interest. The effect of these treatments on purity and potency is important in determining an appropriate dating period for these products.

In the Blood Bank Products Section, the emphasis is on Whole Blood, Single Donor Plasma, Platelets, Cryoprecipitate (Antihemophilic Factor) and the various methods employed in the blood bank to prepare these products with maximum assurance of safety, purity and potency. Random samples obtained in the course of regular inspections of licensed blood banks are thoroughly tested and evaluated for compliance with standards.

The Immunology Section is working on improvements in methods for assay of Rh_o (D) Immune Globulin, a relatively new product. The developing field of tissue transplantation has resulted in the anticipated need for licensing of Anti-Lymphocytic Serum for immunosuppression and the reagents used for determining the compatibility of the recipient with the organ to be transplanted. Prior to licensing these products, it is necessary for the Section to develop test methods, standards and panels of sera for reference

purposes. The Immunology Section is also concerned with an evaluation of pregnancy test reagents.

Blood grouping and typing serums are the responsibility of the Diagnostic Reagents Section. The personnel of this Section are interested in all aspects of these products related to their continued potency after being distributed, improvements in methods of measuring potency, and the reasons for various observed changes in these products on storage.

LABORATORY OF CONTROL ACTIVITIES (LCA)

Mission. The Laboratory of Control Activities has prime responsibility within the Division for performing tests on biological products to determine their conformance to standards of safety, purity and potency. The Laboratory is composed of three sections: Analytical Chemistry, Control Test and Reference Standards.

The Laboratory has the following functions:

(a) To conduct chemical, sterility, general safety, pyrogen, residual moisture and potency tests on biological products submitted for release or in support of license applications. Of all the various licensed products, the approximate number tested by the Laboratory is 300 for sterility, 200 for general safety, 100 for potency, 100 for chemicals, 50 for pyrogen and 40 for residual moisture. This was accomplished by performing 5,243 tests for sterility, 941 tests for general safety, 893 tests for potency, 794 tests for chemicals, 537 tests for pyrogens and 536 tests for residual moisture. Testing is accompanied by review of the manufacturers' protocols with respect to the tests performed by the Laboratory. Applied research is conducted on development and modification of control tests on biological products.

(b) To develop, maintain and distribute biological standards, references, control materials and reagents necessary for the testing of products to determine compliance with published standards. A culture collection is also maintained to support the technical effort of the Division as well as to support control activities as they affect the licensed manufacturers.

(c) In addition to the above, the Laboratory shares responsibility with the other Laboratories in the Division in developing standards and regulations, performing inspections, reviewing license applications and maintaining a technical liaison with the World Health Organization, other national control agencies and recognized authorities in the field of biological products.

Product Testing. Individual lots of licensed products are controlled by the Division through a system of evaluation and release utilizing data submitted by the manufacturer and that developed by the testing program of the Laboratory of Control Activities and other testing areas of the Division. All testing is directed toward the objective of assuring that products meet prescribed standards of safety, purity and potency.

Included in the purity testing of biological products are tests for pyrogenicity, residual moisture and specific chemicals, which also serves as means for evaluating and controlling the safety and stability of such products.

The types of products tested by the Laboratory include antitoxins and therapeutic immune serums, blood and blood derivatives, bacterial vaccines, toxins and toxoids, multiple antigens, viral and rickettsial vaccines, diagnostic substances for dermal tests, antivenins, venoms, bacterial enzymes and allergenic extracts.

In addition to the control testing of biological products within the Division, the Control Test Section of the Laboratory of Control Activities provides regular cooperative pyrogen testing services to the Clinical Center Pharmacy, Blood Bank and Media Department and sterility testing for the Clinical Center Pharmacy and Blood Bank.

A summary of 14,950 tests performed by the Laboratory during a 12 month period (April 1, 1971 through March 31, 1972) in making the determination that products meet prescribed standards is shown in Table 1.

TABLE 1

Samples Tested	Number of Tests Performed				
	Sterility	Safety	Potency	Purity	Total
Products for Release	5,243	941	893	1,867	8,944
Inspection	268	0	4	34	306
Cooperative Service	2,216	0	6	354	2,576
Reference and Standardization	19	1	325	14	359
Complaint	39	27	35	13	114
Research and Development	175	6	1,092	1,378	2,651
Totals	7,960	975	2,355	3,660	14,950

Note: Of the 7,960 total sterility tests, 1,150 (14.4%) were performed for the Clinical Center Pharmacy and 1,020 (12.8%) were performed for the Clinical Center Blood Bank. Also, 35% of all pyrogen tests were performed for the Clinical Center Pharmacy.

Standard Preparations. An integral part of the system of manufacture and control of biological products is the application and use of standard preparations. By the use of such standards, potency of a product can be determined and standardized so as to give increased assurance of consistency from lot to lot. It is the function of the standards program of the Laboratory to provide these standard and reference preparations so that the control program of the Division as well as manufacturers and others engaged in testing may have available the tools to calibrate and evaluate products. It is largely through use of these preparations that those portions of the biologics control regulations relating to potency can be applied.

In order to maintain an adequate supply of standards and references it was necessary to prepare and calibrate during the past year the following lots of materials: 12 antitoxins, 1 serum, 1 vaccine, 6 miscellaneous preparations. In addition to these materials prepared by the Laboratory the following lots of materials were purchased from commercial sources and finished by the Laboratory: 8 virus vaccines, 9 bacterial vaccines, 8 allergenic products, 1 blood typing serum, and 121 hepatitis associated antigen containing sera. A total of 359 tests were required to complete a satisfactory standardization of these materials and maintain surveillance of the stability of all preparations. These tests included such procedures as flocculation reactions, animal protection tests, animal titrations, neutralizations and assorted in vivo and in vitro tests.

The technique for freeze-drying is a key procedure applied in the preparation of standard and reference materials whenever possible in order to increase assurance of stability over long periods of storage. The following numbers of ampules of various preparations were dried during the year.

Cultures	3,209	ampules
Serums	1,267	"
Viruses	40	"
Plasma	4,467	"
Total	8,983	"

The following standard and reference preparations, consisting of a total of 1,115 separate shipments were distributed to manufacturers, P.H.S. and state health laboratories, universities, foreign manufacturers holding U.S. licenses and qualified foreign laboratories.

Australia Antigen	14,021
Antitoxins	486
Serums	4,950
Vaccines	2,498
Toxins	531
Bacterial & Viral Cultures	282
Allergens	425
Viruses	1,713
Antigen E	53

Opacity Standard	90
Globulins	329
Blood Products	920
Miscellaneous	1,348
Total	27,646

Approximately 120 different standards, references, and related control preparations are maintained for distribution.

IV. Related Activities

In addition to the effort outlined above, the laboratory staff also participated during the year in a number of ancillary activities directly related to control which accounted for a substantial segment of its working time.

(a) Members of the professional staff of the Laboratory were members of 66 committees for the review of product and establishment license applications submitted to the Division. In addition 30 reviews of submissions to Investigational New Drug (IND) applications were made.

(b) The Laboratory staff participated in 60 of the on-site inspections required to be performed annually on all licensed establishments. Approximately 69 man days of work were consumed during the year in this effort.

V. Collaboration with World Health Organization

The Laboratory of Control Activities works closely with the biological standardization program of the World Health Organization. Working with Dr. Margaret Pittman (retired) a collaborative study for WHO was performed on opacity preparations. Serving as a reference testing laboratory for cholera vaccine for WHO, four lots of vaccine have been tested. These are samples of vaccine produced in various foreign countries. The World Health Organization uses this method of surveillance to evaluate the quality of vaccines produced in countries having no internal national control program. The quality of vaccines produced in such countries has a direct bearing on the safety of U.S. citizens working and traveling abroad.

VI. Other Activities and Honors

Edward B. Seligmann Jr., Ph.D.	Panel Member of the World Health Organization Expert Committee on Biological Standardization.
	Temporary Advisor for Formulation of International Requirements for Rabies Vaccine, World Health Organization
	Member FDA Task Force on Use of Antibiotics in Animal Feeds
	Member of U.S. Pharmacopeia Advisory Panel on Microbiological Attributes and Procedures

NIAID, Collaborative Research, Research Resources Branch, Consultant for Freeze-drying and Reagent Packaging Contracts

Member of PHS Postal Service Advisory Committee

PHS Observer for Destruction of Biological Warfare Agents

Lee J. Stevan, M.S. — Member of the Executive Committee of the Interagency Botulism Research Coordinating Committee

Research and Development. While the primary function of the Laboratory of Control Activities is concerned with various aspects of controlling the safety, purity and potency of biological products, a substantial research program is conducted in order to provide a sound basis for the testing program and selection of preparations for use as official standards and references. The following are summaries of some of the research conducted by the Laboratory personnel during this fiscal year.

(a) Opacity Standard: The U.S. Opacity Standard which is a suspension of fine glass particles in water, is used to adjust bacterial vaccines to required concentrations and to adjust the cell content of challenge suspensions used in potency tests. Currently, these comparisons are determined in different types of instruments that are designed to measure density. Because of its composition there are inherent problems concerning stability and reproducibility of lots having consistent properties. During storage and use, the density of the preparation is gradually reduced by loss of glass particles from the suspension due to adherence to the sides of the container. To overcome these problems, experimental lots have been prepared by the suspension of glass particles in a semisolid resin. Although such a preparation would provide a greater degree of stability, it presents problems of a different nature. Because heat must be applied, to a limited degree, to liquefy the resin, the mechanics of particle suspension, mixing and dispensing add to the difficulty of preparing a reasonably large lot having a neglible variation in optical density from tube to tube. An additional problem to be resolved, concerns the method of transferring the resin preparations from the container in which it is distributed into suitable colorimeter tubes designed specifically for use in each of a variety of instruments.

The possibility of using the current type of preparation in determining density by visual means i.e., without the use of an instrument, has also been evaluated. Difficulties have been encountered in this method due to the presence of color that tends to influence density comparisons made by eye. Color does not interfere with determinations by instrument.

The U. S. Opacity Standard, as it is presently distributed, has continued to be used successfully for a number of years in the production of bacterial vaccines and preparation of challenge suspensions. Until the problems encountered in preparing a different type of standard are resolved, the

U. S. Opacity Standard will continue to be prepared by the same proven method and distributed for use in comparative density determinations by instrument.

(b) Sterility Testing: Several aspects of sterility testing are being studied with the object of improving the validity of sterility tests and reducing the time, volume of medium and cost involved in performing each test. New and modified media are being studied using selected microorganisms having peculiar growth characteristics. Methods for using membrane filtration for testing a variety of products are being developed. An improved cutter for dividing the membrane in two exactly equal portions was developed. An instrument capable of detecting contamination automatically in biological samples was investigated. Although the numerous mechanical and electronic problems which developed were quickly and efficiently resolved, the unexpected biological problems which developed could not be resolved without redesigning the instrument and/or developing an acceptable radioactive medium. Since neither of these areas of study is within the purview of this Laboratory, the instrument was returned to the manufacturer. In addition, the instrument was not capable of detecting anaerobic organisms or non-glucose fermenting organisms within a period of time shorter than that required by routine laboratory techniques.

(c) Coccidioidin: Under two contracts and with LCA and LBP, a collaborative study is in progress to develop a satisfactory reference preparation for coccidioidin and a suitable laboratory potency test. This work will be correlated with responses in man.

(d) Typhus Vaccine: Vaccines prepared in a series of four serial two-fold dilution levels of activity have been obtained under contract with a licensed manufacturer. The four preparations have been tested in laboratory studies for safety, potency and purity. They possess an extremely high level of purity. A clinical study with these vaccines has been initiated at Camp Lejeune, North Carolina and sera from the first phase of the study are being titered. The project on Typhus Vaccine is being conducted in collaboration with the Laboratory of Virology and Rickettsiology.

(e) In Vitro Test for Diphtheria Antitoxin: Studies are in progress on the use of cell cultures for titrating diphtheria antibody. The results look promising and the procedure offers the possibility of improved sensitivity over that of the present animal test.

(f) Pyrogen Testing: Studies are in progress evaluating the Limulus test for endotoxin as a possible rapid in vitro test for pyrogens in biological products. The test is extremely sensitive for endotoxins, is rapid, and is inexpensive. It is very promising as a substitute for the expensive and time consuming in vivo pyrogen test. However, there are a number of critical details of the test procedure which must be studied and standardized before it will be suitable for routine use. So far no material has been found that is pyrogenic to rabbits and not detected by the Limulus test.

Laboratory of Pathology (LP)

The program of the Laboratory of Pathology basically has been that of the study of the pathogenesis of infectious diseases and immunologic processes. This research program is presently quite broad and reflects the diversity of interests of the scientific personnel in the laboratory. These interests include cytology, virology, immunology, oncology, histology, and primate biology. All of these fields relate, however, to projects designed to meet the overall needs of the Division in regard to the safety and efficacy of biological products both new and old.

Research Activities. Studies of the various candidate agents of human hepatitis have continued with the major emphasis during this year on serum hepatitis or hepatitis Type B. Drs. Chisari, Kirschstein and Lorenz and Mr. McGrath of LP along with Dr. Barker, LVI, and the staff of Hazelton Laboratories have induced evidence of infection in six chimpanzees after inoculation of human serum previously found infectious for man. Infection is usually manifest by the development of circulating hepatitis B antigen (hepatitis associated antigen) 3-5 months after inoculation and/or of circulating antibody. In two animals, evidence of liver damage also was found. Histologic changes of acute viral hepatitis were seen. Thus, an animal model of hepatitis, as a disease entity, has been developed, and expanded studies of the pathogenesis are in progress. (Project No. 314, p. 33).

Mr. McGrath has studied liver biopsy specimens from the chimpanzees and has found "virus-like" particles in the nuclei of hepatocytes similar to those seen in human hepatocytes in liver biopsy material obtained from patients with serum hepatitis. (Project No. 317, p. 41).

Drs. Petricciani and Lorenz and Mrs. Hopps, LVI, in collaboration with the work performed under Contract No. NIH-69-100 have continued the studies of the nonhuman primate diploid cell line. The cell line appears to be very promising in regard to the growth of those vaccines from which vaccines are currently being produced or are contemplated in the future. Dr. Petricciani has developed a sensitive system of evaluation of the oncogenic potential of primate cells in cultures using primates treated with specific anti-lymphocyte globulin prior to inoculation with the cell suspensions to be tested. Cultured cells from known human malignancies such as KB cells and HeLa cells form tumors in monkeys so treated whereas neither primary monkey cells or the diploid line will do so. (Project No. 310, p. 18).

Dr. Singer has been studying the respective roles of immunity and interferon production in respiratory infections. Mice treated with immuno-suppressive drugs develop a more virulent, lethal infection after instillation of Mycoplasma pulmonis into the nares than do untreated mice. On the other hand, similarly treated mice infected with influenza virus have less illness than untreated animals suggesting that immune response may enhance certain respiratory infections while suppressing others. (Project No. 313, p. 28).

The role of herpes viruses in the development of neoplasms has been investigated by Drs. Kirschstein and Lorenz in collaboration with Dr. Rabson of NCI. Herpesvirus saimiri, a naturally occurring herpesvirus of squirrel monkeys, causes the development of lymphoma in the marmoset. Evidence of the presence of the virus or the viral genome can only be demonstrated by sensitive coculti-vation techniques. (Project No. 44, p. 9).

Dr. Shabo has applied the hydrogen peroxidase labeling technique to the study of viral antigens in cell culture and in animals (Project No. 202, p. 30).

The laboratory staff participate in a large program of collaborative research projects which are underway not only with other laboratories in the Division but with NCI, NIAID, NINDS, and NIMH. Some of these projects are specifically related to control activities of the Division and others have a tangential relationship. This collaborative research program as all other research activities is under the direction, supervision, and coordination of the Chief of the Laboratory.

Collaborative (Contracts) Activities. The Laboratory of Pathology plays a very active role in the collaborative program of the Division, since most if not all contracts have aspects relating to pathogenesis and require pathologic interpretation. The Chief of the Laboratory of Pathology in the past, worked very closely with the various project officers, the Contracts Operations Officer, and the Director, DBS in helping to plan and execute various projects and to review progress reports of those contracts already in existence. She also reviews histologic material from contract work as necessary. In order to formalize this relationship, the Chief of LP was appointed Assistant Director (Research Contracts).

Dr. Petricciani has served as Project Officer for four contracts concerning the development of diploid cell strains from subhuman primates and another contract concerning survival of donor white blood cells in recipients. He also served as an Assistant Project Officer for one contract while Dr. Lorenz served as Assistant Project Officer for three contracts. Dr. Chisari serves as Assistant Project Officer for one contract. Dr. Singer serves as project officer on one contract.

Laboratory of Virology and Rickettsiology

During the fiscal year, continued emphasis was placed on the development of a program which would coordinate straightforward control with control-related research responsibilities. Progress has been made in phasing out old programs efficiently and developing new meaningful ones.

Mission. The Laboratory encompasses a broad program of research concerning the prevention and treatment of viral and rickettsial infections in man. Specific emphasis is placed upon problems as they relate to the mission of DBS, such as problems concerned with vaccine safety, potency, efficacy and standardization. Constant liaison with the Division's other Laboratories achieves a balance in the harmonious execution of the DBS control and research responsibilities.

Influenza Vaccine. A major portion of the Laboratory is devoted to the execution of the Division's responsibility with regard to influenza vaccine. A constant effort is required (1) to maintain a competent Respirovirus Unit for the study of influenza viruses and other viral agents etiologically associated with respiratory tract infections of man; (2) to maintain a reference collection of infectious viral antigens and antibody reagents of influenza and other respiroviruses including those of potential importance as vaccine constituents; (3) to review and analyze manufacturers' protocols and to perform potency tests on current influenza vaccines; and (4) to improve serologic techniques for immunogenic potency testing of influenza vaccines.

We developed a quantitative radial immunodiffusion test to detect soluble residual host protein in chicken embryo-derived vaccines. Smallpox, yellow fever, and 1967 and 1968 influenza virus vaccines produced by the Sharples process had significant quantities of residual soluble host protein; 1969 influenza virus vaccines, produced by processes designed to purify the viral antigen, either had no detectable residual protein or borderline (1 mcg/ml) quantities. This immunodiffusion test will be employed on all influenza vaccine lots submitted for release in the hope that we will make progress in eliminating residual host protein in inactivated influenza vaccines.

Results of serological testing of nonhuman primate sera obtained over a four year period showed a high incidence of antibodies to influenza virus strains of the H2 and H3 hemagglutinin subtypes. This would indicate that outbreaks of type A influenza virus infection occurred in certain primate species and suggest another possible reservoir for influenza virus in nature.

Volunteer studies were performed to make comparative studies on monovalent influenza vaccines produced by different manufacturers and administered by alternative routes. The incidence of reactions was lowest among volunteers receiving the more highly purified vaccines, such as zonal purified, ether-extracted hemagglutinin and filter-purified Sharples vaccines. Interestingly, however, there was no apparent relationship between reactogenicity and measureable avian antigen and protein nitrogen.

Analysis of laboratory data showed that certain tests (i.e., CCA and neuraminidase) were quite reproducible; whereas, considerable differences were observed in the results of measuring virus particle count and protein nitrogen. Our results, however, did show that decreased protein nitrogen was associated with increased vaccine purity. Additionally, residual host protein was found to be reduced but not eliminated in these newer vaccine preparations.

More highly purified influenza virus vaccines, whether consisting of intact virions or ether-degraded hemagglutinin, induced antibody responses approximately equivalent to the cruder more reactogenic Sharples-type vaccine preparations. The presence of aluminum phosphate adjuvant, while increasing reactogenicity, did not improve the antibody response. Comparable serologic responses were found for chicken embryo chorioallantoic-derived vaccines and for vaccines prepared in bovine kidney cell cultures. Intramuscularly administered vaccine was found to be as effective as vaccine administered subcutaneously and caused less local reactogenicity. The demonstration of an inverse relationship between the pre-immunization antibody titer and the serologic response to vaccine would appear to justify the continued immunization of non-immune individuals.

Surprising as it may seem, even after influenza vaccine has been in continuous use for almost 25 years, its efficacy is much in doubt. It is our feeling that this doubt has arisen from the confusion of a multitude of contradictory reports in the literature. A critical review of this literature has revealed that most investigators have not appreciated the fact that until most recently we lacked reliable methods for quantitating the antigen content of vaccines. We strongly feel that clinical evaluation studies must be supported by a critical laboratory analysis of antigen content, and a lack of knowledge of the latter precludes any meaningful conclusion of efficacy studies in man. Our influenza vaccine program was initiated with the development of a reliable and reproducible method of quantitating vaccine antigen. With this solid foundation we are progressing to volunteer studies. We feel that our methodical approach will allow us to make some definitive conclusions on influenza vaccine efficacy.

Yellow Fever Vaccine. The DBS yellow fever project was established in April 1966 as a coordinated research program involving several laboratories in the Division. Scientific profits have been realized from this team approach to problem solving.

Studies of the antigenicity and safety of the avian leukosis virus -- (ALV) --free yellow fever (YF) vaccine primary seed have been completed. The safety and antigenicity of the ALV-free YF virus strain in monkeys and man have been established. A recommendation has been made to adopt the use of the new ALV-free YF primary vaccine seed.

Progress has been made towards the development of a cell culture derived YF vaccine. Adequate virus titers were achieved in virus-infected primary chicken embryo cell cultures. Neurovirulence studies on several cell culture-adapted virus strains are currently under way. Plans include antigenicity studies in monkeys and a clinical evaluation in man.

The finding of a suitable yellow fever virus stabilizer will enable us to lyophilize cell culture-derived yellow fever vaccine, package it in rubber-sealed ampoules, and store the vaccine at convenient temperatures.

Studies were performed to determine the effect of graded doses of 17D vaccine upon viremia, antibody response and efficacy in rhesus monkeys. There was a direct correlation between dose of inoculum and the level of antibody as measured by the HI and plaque neutralization (PN) tests. Optimal antibody responses were obtained with a vaccine dose as small as 3.4 Dex (decimal exponent) weanling mouse intracerebral LD_{50}. The significance of the PN antibody titer was extended by results of direct challenge studies. Both PN antibody titers and conversion rates were positively correlated with immunity. A neutralization index of at least 1.0 Dex reflected immunity to challenge with "wild type" virus. The HI and CF antibody titers and antibody conversion rates were not meaningfully related to vaccine dosages or immunity.

Egg Vaccines. The developing chicken embryo remains one of the most versatile substrates for vaccine manufacture. In order to maintain vaccine purity, it has been necessary to survey avian flocks for potentially troublesome microbial infections and to maintain chicken flocks which are free of known viral, bacterial and mycoplasma contaminants. The Avian Leukosis and Immunofluorescence Units of this Laboratory provide the DBS with the competency to deal with potential problems in this area.

Recently, we have been able to use the fluorescent antibody (FAB) test to detect the virus causing Marek's disease, a contagious neuroproliferative disorder which is prevalent in chicken flocks in this country. From what is already known about Marek's virus, it is highly unlikely that this virus could survive the commonly employed procedures for preparing live virus vaccines. We will soon be able to examine finished vaccine and remove the speculation concerning the potential contamination of egg derived vaccines by Marek's virus.

The Avian Leukosis Unit of this Laboratory has been responsible for monitoring the NIH ALV-free chicken flock to insure that the flock remain free from these viruses. From our collaborative studies with a USDA geneticist we recently discovered that the NIH flock was not homogeneous genetically and that, by and large, the flock was resistant to avian leukosis subgroups B viruses (i.e., C/B). We have been able to identify and segregate some chickens with respect to their genetic susceptibility. A system of cross-breeding has been established and the embryos of trap-nested eggs are currently being screened to determine their genetic status. It is our hope that, eventually, we will be able to establish a flock which will be susceptible to viruses of all subgroups (i.e., a C/O flock). This will enable us to perform more easily our control responsibility to insure that vaccines produced in chicken embryo derived tissues are free from contaminating avian leukosis viruses. The maintenance of an avian leukosis virus genetically susceptible flock is a time-consuming, lack-lustre chore which is not fully realized by egg users, but which is nevertheless singularly necessary for certification of a "gold-bar" flock.

Rickettsial Vaccines. As with most killed antigens, it is difficult to measure the potency of rickettsial vaccines. Emphasis was directed towards developing a candidate lot of inactivated Typhus Vaccine for use as a reference antigen. Lots of commercially produced Typhus vaccine believed suitable for a reference vaccine have been obtained and tested by the Laboratory of Control Activities (LCA), DBS and by the manufacturer. The four candidate lots of vaccine have passed the safety, purity and potency tests ordinarily required for licensing of Typhus Vaccine. Clinical trials have been initiated to evaluate the reactogenicity and antigenicity of these preparations in volunteers.

Arbovirus Studies. With abolishment of NIAID's Laboratory of Tropical Virology, the Arbovirus Unit of our Laboratory became the only facility on the NIH reservation with the professional and technical competency capable of performing studies on arboviruses. The working collection of viral and antibody reagents has expanded considerably. Present reagents representing a number of virus strains of most of the major and minor arbovirus groups are available and being utilized for routine and special studies.

Hemorrhagic Fever Studies. Previous reports described this Laboratory's isolation of the heretofore unknown virus of simian hemorrhagic fever which was responsible for a devastating epizootic of hemorrhagic fever among colonies of rhesus monkeys at the Primate Quarantine Unit, LAB, DRS (Fall, 1964), and at the Sukhumi Institute of Experimental Pathology and Therapy, USSR (July, 1964).

Our studies have revealed that the isolation of SHF virus strains in cell culture is dependent upon the use of a susceptible MA-104 cell strain and that the ability of such strains to support the replication of these viral agents may vary. Using this information we were able to isolate a viral agent in cell culture from materials derived from the Sussex/69 epizootic.

Viral Pathogenesis Studies. A new class of unusual agents found to cause diseases in man (kuru, subacute sclerosing panencephalitis) and animals (scrapie, visna, maedi) show unusual properties, such as long latent incubation period of months to years, protracted disease course, and pathologic changes unlike those of acute viral diseases. Studies with this new class of infectious agents and possible contaminants of biological products will provide DBS with the competency to develop testing and monitoring techniques.

Monkeys infected with the neuropathic measles virus developed a subclinical progressive encephalitis. Immunization preceeding the infection with the neuropathic virus prevented encephalitis. However, once encephalitis was initiated in non-immune animals, attempts to stimulate cellular immunity with antigen incorporated into complete Freund adjuvant were of limited beneficial effect. In partially immunosuppressed animals, the disease took a lethal course 2-3 weeks after infection. Stimulation of the immune response in those animals prevented the lethal outcome but did not eliminate the virus from the CNS. Partly immunosuppressed monkeys, superinfected with pantropic measles virus in the early stage of measles encephalitis, developed a hyperacute lethal encephalitis.

Additional studies were performed to evaluate the circumstances under which measles virus displays neurotropic properties. It was found that on initial passaging of the hamster neurotropic measles virus strain in cell culture, there ensued a slowly progressing infection characterized by cell-to-cell spread and production of large syncytia. No infectious virus was released into the medium and the infected cells lacked specific determinants for hemadsorption on their surface virus. Plaque purified giant cell variants displayed different degrees of neurotropism indicating that the capability of the virus to proliferate in nervous tissue and giant cell CPE are independent properties of the virus. However, the association of these two properties combined with a defective replicative cycle appeared to be prerequisite for high neurovirulence as evidenced by encephalitogenicity in monkeys.

Knowledge of the biological properties of neurotropic strains as well as the character of the host reaction are important for a better understanding of potential preventive or curative measures.

Cell Culture Studies. A program of research and development in connection with production of cell cultures for the Division is actively pursued. Studies involved: (1) developing new and improved techniques and methods for large-scale production of cell cultures; (2) developing additional cell culture systems as potential substrates for vaccine purposes; (3) studying selected biochemical parameters of cells in culture as may be related to viral susceptibility; and (4) studying factors which may influence somatic variation and modulation in cell culture systems.

As a result of some of these studies, many practical improvements have been introduced into routine cultivation of cells. Increasing emphasis is being given to studying the general biology and immunobiology of cultured cells.

Laboratory of Viral Immunology

The program of the Laboratory of Viral Immunology is research oriented and directed so as to meet current and anticipated needs of the Division in the area of viral diseases. The Laboratory conducts direct research, assists in contract-supported collaborative research management and is involved in the review of data submitted to the Division in its capacity as a regulatory agency. These reviews deal with existing biological products, license applications for new products and information submitted in connection with investigational new drugs (biologics). Laboratory work involves product testing and development of new test procedures as well as more widely ranging investigational efforts. Research conducted by the LVI tends to be applied in nature and product or disease oriented. The conduct of clinical trials is an integral part of the program.

The strength of the Laboratory's operation lies in the bringing together over a period of years of a highly qualified and motivated staff capable of promoting the necessary interation between regulatory needs and research developments. Senior personnel are familiar with regulatory requirements and manufacturing practices and have product testing and other control responsibilities. These same people direct research and are recognized as experts in their scientific fields. The investigations yield data needed in the regulatory program. Equally important, by virtue of their investigations, our staff is able to maintain lines of communication with other laboratories and to analyse critically information generated by other scientists that may be of current or future consequence in controlling biologics. Our staff received a number of honors during the year. Several served as consulstants to expert committees and were invited to participate at international and national conferences. Two LVI investigators received one of America's top research prizes, the Joseph P. Kennedy, Jr. Foundation International Award for Distinguised Scientific Research in recognition of their contributions to the control of rubella.

Selected items from the program are discussed in the following paragraphs.

Hepatitis. Approximately one third of the total effort of the LVI is committed in this area. The work is following two lines: far ranging research on the disease (and viruses causing the disease) and targeted efforts to insure that pertinent new knowledge is immediately translated into the regulatory program to improve the safety of blood and blood-derived products. Human blood, has in the past, often contained hepatitis B (serum hepatitis) virus and recipients were thus at risk of contracting this serious and sometimes fatal disease. Only recently as a result of a substantial research breakthrough has it been possible to develop tests capable of detecting the virus. These tests are complex immunological procedures, yet through the targeted efforts of LVI scientists and other investigators the procedures

have been perfected to the point of having wide application. The DBS has developed standards and rated the sensitivity and reliability of each of the methods. Our staff has been involved in extensive comparative testing with pharmaceutical manufacturers and as a result of this rapid translation of research into the regulatory program, there are now nine manufacturers licensed to produce diagnostic kits for use in blood banks and other laboratories. The use of these new standardized reagents is expected to reduce the incidence of post-transfusion hepatitis caused by this virus by at least 50%. The broader hepatitis research efforts include a search for still better methods of virus detection, a study of the role of antibody and immunity in preventing the disease and an investigation of the importance of this virus in other serious illnesses such as liver cancer, arthritis (and other "autoimmune" diseases) and chronic liver failure. Preliminary work indicates that many persons with the above listed diseases are indeed carriers of the virus. Our hepatitis investigations serve as an example of the multiple approaches taken in solving important problems. There is a major "in house" research committment, a broad collaborative (contract-supported) effort supervised by our scientists and extensive collaboration in testing and exchange of data with pharmaceutical manufacturers and investigators in universities and in other government laboratories. Our personnel have assisted in drafting regulatory requirements, been involved in product testing, developed standards, participated in important research discoveries and served on numerous committees as experts in their field. Their activities have ranged from basic laboratory research to clinically oriented epidemologic studies in hospitals and blood banks of transfusion-associated risks to blood recipients.

Viruses and Cancer. All evidence points to viruses as a major cause of human cancer. In the veterinary field the identification of a herpesvirus as responsible for a common malignancy of chickens has now been followed by the development of a virus vaccine that has effectively prevented these cancers. The implications of these and other recent discoveries in human medicine have been widely appreciated as can be judged by the enormous present and projected committment of public funds to cancer research. Moving with this important field and anticipating the possible future development of new anti-cancer biological products, the LVI has increased its efforts in the herpesvirus and viral oncology area from approximately 10% of total effort four years ago to approximately 30% today. In the current reporting year LVI investigators have shown the genome of EB virus (the herpes virus recovered from African lymphoma tumor tissue) as it exists in a silent state in human white blood cells can be activated to a replicating virus cycle by certain types of drug treatment. Also our scientists have discovered an entirely new family of herpesviruses of subhuman primates that like EB virus appear to associate with the white blood cells of the animal and induce a life-long carrier state. There are many implications of this work, one is that of using subhuman primates and these new viruses as models for the study of herpesviruses and cancer and herpesvirus immunity. In recognition of the quality of the research effort of the laboratory, one member of our staff is a member of the Developmental Research Working Group of the Special Virus Cancer Program of the National Cancer Institute.

Arthritis, Autoimmune Disease and Long-Term Effects of Virus. There is speculation that viruses may be important causes of autoimmune diseases of man. The postulation is that after an initial infection, viral antigens might persist and years later trigger destructive antibody responses against the human tissues harboring the viral antigens. Rheumatoid arthritis could be an example of this situation. It is known that infection with either natural rubella virus or attenuated (vaccine) strains of rubella virus is often associated with viral replication in joint tissues and transient evidence of arthritis. The importance of determining the role, if any, of this virus in progressive arthritic conditions, such as rheumatoid arthritis, is apparent. The LVI has been applying the newer tools of virologic study to arthritis over the past several years and to date has found no evidence of association of rubella with chronic arthritis.

A new section of the laboratory is conducting a long-term study of inactivated influenza vaccine in animals. Here the intent is to determine if over a period of time the protective effect induced by vaccine could wane leaving a hypersensitivity that could enhance susceptibility to influenza. This type of investigation is important since this unexpected and undesirable effect has been noted following immunization with killed measles and killed respiratory syncytial virus vaccines. It should be noted that no adverse effects of this type has been associated with influenza vaccine use. Nevertheless, the initiation of studies searching for unexpected long-term effects of viruses and viral vaccines is extremely important and it is our intent to increase the scope of our long-term investigations.

Clinical Studies. Clinical trials to confirm and extend data aquired by manufacturers are an integral part of our program. Our laboratory has conducted experimental studies with each of the live virus vaccines licensed by the Division since 1960. These include the vaccines for poliomyelitis, measles, mumps and rubella and the new combinations of certain of these vaccines. Normally our clinical trials preceed the development of regulations and license but may include vaccines in routine use if there is a need for additional data. During the current report period LVI medical officers have conducted investigations with all four of the above listed live virus vaccines to obtain information relating to potency ranges and duration of vaccine-induced immunity.

As part of this work the laboratory developed two new serologic procedures to measure polio and mumps antibody levels (immunity). These techniques employ microtiter methods and as a result require only tiny amounts of human serum, a distinct advantage in measuring the antibody response of infants and preschool children.

Cell Culture Studies. Cell cultures are basic to virtually all research with viruses; consequently many projects in the laboratory involve cell experimentation. During the current year both intramural and collaborative research efforts have been continues on several cell substrates used for vaccine production: monkey kidney, rabbit kidney, duck embryo and human diploid fibroblasts. These studies have increased our knowledge of the chromosomal and other characteristics of these cells and on their freedom from

contamination by infectious agents indigenous to the host source. The laboratory has prepared and used more than 30 distinct types of tissue cells from man, monkeys, rabbits, dogs, mice, rats, swine and hamsters in its research program. These included cells derived from lung, kidney, skin, muscle, joint surfaces, cartilage, bone marrow, blood, liver, spleen, eye, salivary gland and prostate gland. The LVI maintains many of the special cells as permanent reference cultures frozen in liquid nitrogen. These cultures are furnished on request to investigators all over the world.

Special Problems. There are two special problems that should be mentioned since they have an major hearing on current and future performance of the organization. These are: (1) an imbalance between needs and resources (including personnel strength) to meet those needs and (2) our susceptibility to greater damage than benefit as a result of the continuing critical dialogue concerning the performance of the DBS as a regulatory organization. As I see it our most critical needs in the coming year will be in trying to find ways to correct these problems.

Annual Report
of the
Laboratory of Bacterial Products
Division of Biologics Standards
July 1, 1971 through June 30, 1972

The program of the Laboratory of Bacterial Products includes a variety of research projects. Some are designed to investigate basic mechanisms of allergy and infectious diseases, while others involve applied research designed to develop new or improved standards and testing procedures for products subject to license under the Public Health Service Act.

In addition to research, official potency and safety tests are carried out on lots of certain products to determine whether they meet requirements for release. Much time is also devoted to the review of protocols, license applications, amendments, label and IND reviews and in travel to inspect licensed establishments as well as those establishments seeking a license.

Various members of the laboratory serve as project officers or assistant project officers for contracts whose purpose is to supplement laboratory programs.

The Section on Bacterial Vaccines has a new chief, Robert S. Northrup, M.D.

Research Activities:

Allergenic Extracts: The potency assay for extracts of short ragweed pollen is now a practical assay and is being used to study stability and uniformity of commercial and laboratory preparations of such extracts. In this way it may be possible to assign storage and dating periods for products of this type based on the known properties of the product. There is, however, a serious need for additional assay methods so that potency assays and reference preparations can be prepared without the need for isolation of a pure allergen from each source. New procedures for this purpose based on a radioimmune assay are under investigation for this purpose. In addition, a bioassay is under development for bee venom, a product that may have wider use in the future.

Poison Ivy and Poison Oak: Assays for the active principles of those products have been developed and it has been demonstrated in collaboration with a contractor that the active principle can, in fact, reduce the sensitivity of sensitive humans. It should now be possible to apply this information to licensed products. Studies in animals are designed to elucidate the basic mechanisms of sensitization and tolerance.

Mycoplasma: New species of mycoplasma continue to be uncovered. M. primatum was isolated from oral and urogenital tissue of the African green monkey. M. conjunctivae was isolated from outbreaks of keratoconjunctivitis of sheep and goats.

Mycoplasma and acholeplasma continue to be serious problems in the manufacture of biological products since they are frequent contaminants of bovine serum used in cell culture. Their presence is a continual problem to manufacturers of products derived from cell cultures. New and improved methods of isolation and identification are being developed to study this problem.

Bacterial Toxins: Tetanus toxin is composed of at least two components, a neurotoxin and an hemolysin, tetanolysin. Studies on the mechanism of action of the hemolysin revealed that it disrupts lysosomes and platelets releasing enzymes and serotonin.

Biologic Products as Interferon Inducers: The potency of certain biologic products has not been subject to measure; these include mixed bacterial vaccines and house dust extract. Studies in mice now demonstrate that some of these products are interferon inducers, and that house dust extract can non-specifically reduce certain viral infection in mice. It thus appears that these products have a biologic potency that may account for the claims made for them in the treatment of human disease. This is only a beginning, but suggests that much useful work needs to be done with products of this type.

Mycobacteria and Mycobacterial Antigens: BCG continues to be an organism of considerable interest. Although all current strains originated from that isolated at the Pasteur Institute, they have been subcultured many times in many parts of the world. Many of these strains differ in their cultural characteristics, but a thorough analysis of the antigens in culture filtrates established a remarkable similarity among all strains.

Much effort has been expended in improving the specificity of the tuberculin reaction. A new analysis of the culture filtrate antigens of M. tuberculosis and a variety of mycobacteria representing the 4 Runyon groups failed to reveal any antigen unique for M. tuberculosis. It reduces further the probability of finding a completely specific tuberculin test.

Cholera: It has now been demonstrated conclusively that cholera toxoid prepared with formaldehyde is unstable and can revert back to toxin. A cholera toxoid prepared by inactivating the toxin with glutaraldehyde is now under study. This product appears to be stable and immunogenic in rabbits and monkeys.

The role of cholera toxin in inducing the diarrhea characteristic of the disease was investigated. Intestinal loops in monkeys, when challenged with toxin, produced diarrhea fluid. Continued challenge rendered the loop resistant, a condition that seemed to correlate with the development of antitoxin.

Studies on El Tor and Classical strains of V. cholerae showed that their relative sensitivity to sulfamethoxazole and trimethoprim was a useful means of differentiating these strains.

Typhoid Vaccine: The importance of Vi-antigen is still not established, but has now been demonstrated to be a protective antigen in mice. To continue studying this antigen, an improved method of isolation was found that results in an antigen at least 10 times more protective than those previously isolated. A new sensitive and specific method for the assay of this antigen has been developed.

Pertussis: Work has been in progress for some years in developing an improved mouse strain for the pertussis potency and toxicity tests. In cooperation with the Division of Research Services, some mouse strains have been developed that give promise of being better than that currently employed.

Control Activities:

Potency and Safety Tests

The Laboratory of Bacterial Products performed tests on and/or reviewed protocols as follows:

Tests and protocol reviews

Type of product	Purpose	Type of test		Protocols reviewed
		Potency	Safety Toxicity	
Pertussis Vaccine	Official*	110	110	104
	Special	2	3	
Pertussis Immune Globulin (Human)	Official	8		3
Haemophilus influenzae typing serum	Official	1		7
BCG Vaccine	Official	27		30
			Mycoplasma only	
Measles Vaccine	Official		9	57
Mumps Vaccine	Official		1	8
Poliovirus Vaccine	Official		5	29
Rubella Vaccine	Official		2	10
Total		148	130	248

*Tests of samples of lots for release, inspection or license application.

Activities requiring travel status

Type of Activity	Personnel	Locations	Man days of travel
Annual inspections	9	35	62
Contracts	4	13	17
Meetings	12	41	145
Other	2	2	6
Total			230

License applications and amendments, notices for investigation of new drugs (IND), and label reviews

Category	Number	Personnel
License	73	11
IND	29	10
Label Review	66	9

Other Activities and Honors:

Dr. Harold Baer	Committee on Standardization of Allergens, American College of Allergy
	Committee on Specific Therapy American Academy of Allergy
	Diagnostic Skin Test Committee of the American Thoracic Society
	NIAID Research Reference Reagents Branch Ad Hoc Committee on Ragweed Allergens
	International Union of Immunological Societies - Member of Standardization Sub-Committee
Dr. Michael F. Barile	Officer, Section on Mycoplasma, American Society for Microbiology
	NIH Graduate Program, Instructor in Microbiology
	Guest Lecturer, University of Maryland
	Head of Laboratory Animal Mycoplasmas, Working Group WHO/FAO
Dr. Sotiros D. Chaparas	NIH Graduate Program, Faculty Chairman Microbiology and Immunology
	International Working Group for Mycobacterial Taxonomy, Working Member

Dr. Chaparas (cont'd)	American Society for Microbiology, Washington, D.C. Branch, Past President (1971) and member of Executive Board
	Trudeau Institute, Inc., Member Advisory Committee
	International Association of Microbiological Societies, International Committee on Systematic Bacteriology, Sub-committee on Mycobacterium, Corresponding Member
	U.S.-Japan Medical Cooperative Program, Panel on Tuberculosis, Antigen Study Committee, Member
	American Thoracic Society, Membership Chairman, Scientific Assembly on Microbiology and Immunology
	American Thoracic Society, Scientific Assembly on Tuberculosis, Member Ad Hoc Committee on Tuberculin Skin Testing
	Howard University, Guest Lecturer
	George Washington University Graduate School, Examining Committee for two Doctoral Candidates
Dr. Carolyn Hardegree	NIAID, Member of Cholera Advisory Committee
Dr. Charles R. Manclark	American Society for Microbiology, Washington Branch, Secretary and Education Committee Chairman
	International Committee on Bacterial Nomenclature, Sub-committee on Taxonomy of Vibrios, Member
	NIH Graduate Program, Faculty Member, Medical Bacteriology
	Symposium on Recent Developments in Research Methods and Instrumentation, American Society for Microbiology Representative

Dr. Robert S. Northrup	NIAID, Cholera Research Program Coordinator
	NIAID, Laboratory of Clinical Investigation Clinical Consultant in infectious diseases at Clinical Center and National Naval Medical Center, Bethesda
	Georgetown University, Clinical Instructor in Medicine, also guest lecturer in International Health
	WHO, member of emergency consultant team for cholera epidemics
	International Association of Microbiological Societies, 12th International Congress of Microbiological Standardization, Annecy, France - Chairman, Section on Cholera
	Project Officer, various NIAID cholera-related contracts

Serial No. DBS 102
1. Bacterial Products
2. Allergenic Products
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Immunology of the Delayed Contact Sensitivity Induced by Catechols Related to and Contained in Poison Ivy Extracts

Previous Serial Number: Same

Principal Investigators: Harold Baer, Ph. D.
Mitchell H. Friedlaender, M.D.

Other Investigators: C. J. Maloney, Ph. D. (OD)
Philip R. B. McMaster, M.D. (NIAID)
Richard A. Johnson, M.D. (NIAID)

Cooperating Units: Office of the Director
National Institute of Allergy and Infectious Diseases

Man Years

Total:	2.5
Professional:	1.25
Other:	1.25

Project Description:

Objectives: (1) To examine the composition and to establish a method for standardization of poison ivy extracts and related plants such as poison oak and sumac. (2) To determine the relationship of structure of catechols to their effectiveness in inducing delayed contact sensitivity and immune tolerance. (3) To study the mechanisms of delayed contact sensitivity and immune tolerance, and the role of the regional lymph node in these processes. (4) To study the relationship between the physicochemical properties of haptens and their ability to induce sensitization and tolerance.

Methods Employed: (1) Guinea pigs sensitized to various synthetic catechols were used to evaluate the relationship of catechol structure to immunologic specificity and to their ability to induce immune tolerance and delayed contact sensitivity. (2) Sensitizing chemicals were applied to the skin of guinea pigs in such a manner as to study the importance of draining lymphatics and blood vessels in the induction of sensitivity and tolerance. (3) Chemicals which induced sensitization and tolerance were also applied to the skin of germfree guinea pigs to determine their ability to develop

delayed contact sensitivity. (4) Chemicals with different physicochemical properties were applied to the skin along with various types of inflammatory agents to determine the importance of inflammation in the processes of sensitization and tolerance. A histologic study of the reactions induced by these procedures was also made. (5) Dilutions of the 4 components of urushiol were applied to the skin of sensitive humans to determine their relative reactivity.

Major Findings: (1) Sensitization does not require the presence of intact lymphatic drainage. (2) Lymphatic drainage does play a role in the induction of tolerance to simple chemicals. (3) Two major groups of simple chemical sensitizers exist; those which are good sensitizers when applied to the skin, and those which are primarily toleragens by this route. (4) The inflammatory properties of simple chemicals influence their ability to induce sensitization. (5) Pentadecylcatechol is the most unreactive component of poison ivy urushiol, the diolefin being the most reactive. (6) Germfree guinea pigs were capable of developing delayed contact sensitivity and immunologic tolerance to simple chemicals.

Significance to Bio-medical Research and the Program of the Division: (1) These studies are providing information on the mechanisms of sensitization and immune tolerance. They may reveal how alterations in the physical and chemical properties of antigens, as well as their method of administration, may influence their ability to sensitize or induce tolerance to allergens. (2) The low reactivity of pentadecylcatechol in poison ivy sensitive people probably explains why previous attempts to use this material for hyposensitization failed. Whole urushiol, containing large amounts of the unsaturated components, should be more effective.

Proposed Course of Project: The project will be continued until all major objectives have been reached.

Honors and Awards: None

Publications:

Godfrey, H. P. and Baer, Harold. The effect of excision of the site of application on the induction of delayed contact sensitivity. J. Immunol. 107:1643-1646, 1971.

Friedlaender, M. H. and Baer, H. Immunologic Tolerance: Role of the regional lymph node. Science (In press)

Godfrey, H. P. and Baer, H. Antibodies Produced after Immunization with substituted Meta-Dinitrobenzenes. J. Immunol. 106:1201-1212, 1971. (Previously reported as "In press.")

Baer, H. and Godfrey, H. Absorption through the skin of sensitizing and non-sensitizing substances related to the active principle of poison ivy. *In* Montagna, W. and Billingham, R. E. (Ed.): Immunology and the Skin - Advances in Biology of Skin, Vol. XI. New York, Appleton-Century-Crofts, 1971, pp. 161-165.

Johnson, R. A., Baer, H., Kirkpatrick, C. H., Dawson, C. R. and Khurana, R. G. Comparison of the contact allergenicity of the four pentadecylcatechols derived from poison ivy urushiol in human subjects. Allergy and Clin. Immunol. 49:27-35, 1972.

Serial No. DBS 119
1. Bacterial Products
2. Allergenic Products
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Standardization of Allergenic Extracts

Previous Serial Number: Same

Principal Investigators: Rudolph M. Franklin, M.D.
Harold Baer, Ph. D.

Other Investigators: Michael Hooton
Frank Malik

Cooperating Unit: None

Man Years

Total:	1.0
Professional:	0.5
Other:	0.5

Project Description:

Objectives: (1) To determine a method for quantitatively measuring allergens in whole allergenic extracts. (2) To investigate classes of immunoglobulins combining with whole allergenic extracts. (3) To measure the stability and quantities of antigen E in ragweed and related pollen extracts. (4) To develop laboratory procedures for measuring the potency of allergenic extracts that are not based on the measurement of a single purified allergen.

Methods Employed: (1) Measurement of antigen E by radialimmunodiffusion following storage of extracts under varying conditions. (2) Use of acrylamide gel electrophoresis to study changes that occur in pollen extracts. (3) In vitro degranulation of normal rat peritoneal mast cells with allergenic extracts after sensitization with specific reaginic sera. (4) Fluorescein or I^{125} labeled allergenic extracts, (a) use these labeled extracts in allergen binding techniques, (b) determine degree of inhibition of specific binding of labeled allergenic extracts by unlabeled allergenic extracts and correlate with amount of allergen in the unlabeled extract, and (c) by co-precipitation determine which immunoglobulin classes bind allergens.

Major Findings: (1) The antigen E content of concentrated extracts of short ragweed stored continuously in the refrigerator drops off slowly over a period of one year. (2) At elevated temperature the antigen E content falls off in days and may be completely gone in 20 days. (3) Fifty per cent glycerol retards the rate of loss of antigen E. (4) Extracts of pollen from plants related to short ragweed may contain antigen E as measured by radial-immunodiffusion. (5) In vitro degranulation of rat peritoneal mast cells occurs in a non-specific fashion following contact with heterologous serum. The test does not appear useful for studies with allergenic extracts. (6) Fluorescein conjugates of ragweed allergenic extract were prepared and shown to retain their antigenicity. Incubation with specific reagenic sera as well as non-reagenic sera (human and rabbit) have so far provided inconclusive results. (7) I^{125} labeled ragweed extract has been shown to retain its antigenicity. Co-precipitation and autoradiography experiments are in progress.

Significance to Bio-Medical Research and the Program of the Division: Presently no measure of potency for allergenic extracts is required by manufacturers of such products. It is anticipated that the present studies will lead to a simple, reliable and accurate test for this purpose. In addition these studies should lead to a further understanding of the nature of allergens and allergen-specific immunoglobulins.

Proposed Course of Project: (1) Continued study of stability and quantitation of the antigen E content of extracts in which this antigen can be measured. (2) Use of I^{125} labeled allergens in allergen binding experiments.

Honors and Awards: None

Publications: None

Serial No. DBS 206
1. Bacterial Products
2. Allergenic Products
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Studies on the House Dust Mites D. farinae and D. pteronyssinus

Previous Serial Number: Same

Principal Investigator: Harold Baer, Ph. D.

Other Investigators: None

Cooperating Units: None

Man Years

Total:	0.2
Professional:	0.1
Other:	0.1

Project Description:

Objectives: (1) To study the antigens of mites. (2) To determine whether commercial house dust extracts contain mite antigens and whether the presence of these antigens can be correlated with the reactivity of these extracts when injected into the skin of allergic humans.

Methods Employed: Mites were obtained from Ohio State University where they are being cultured under a DBS contract. Extracts were prepared and the antigens studied by immunization of rabbits to produce precipitating antisera. Antigens were also sought by the use of PCA and inhibition of PCA tests.

Major Findings: (1) There are many antigens in both D. farinae and D. pteronyssinus. (2) These species are antigenically different but share some antigens. (3) Some but not all commercially prepared house dust extracts contain mite antigens.

Significance to Bio-Medical Research and the Program of the Division: It has been established that mite allergens are a component of most, but not all, allergenic extracts of house dust. This information, together with clinical studies carried out in other laboratories, demonstrates that mite antigen is frequently a component of house dust extract, but is not the only or even a major allergen of such extracts. The mite allergen is therefore of no greater significance than other casual allergenic components of house dust extract.

Proposed Course of Project: No further work is contemplated.

Honors and Awards: None

Publications: None

Serial No. DBS 300
1. Bacterial Products
2. Allergenic Products
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Studies of Honey Bee Venom

Previous Serial Number: None

Principal Investigators: Rudolph M. Franklin, M.D.
Harold Baer, Ph. D.

Other Investigator: Frank Malik

Cooperating Units: None

Man Years

Total:	1.0
Professional:	0.5
Other:	0.5

Project Description:

Objectives: (1) To investigate biological activities of honey bee (Apis mellifera) venom. (2) To determine antigenicity of components of bee venom. (3) To investigate interactions of bee venom with serum components in normal and bee sting allergic people.

Methods Employed: (1) By intravenous injection, determine LD_{50} of venom in mice. (2) Determine the amount of bee venom necessary for the hemolysis of non-sensitized sheep red blood cells. (3) By preincubating venom with normal and immune sera, to determine variations in biological activity by hemolysin assay and mouse LD_{50}. (4) By immunochemical methods, determine interactions of bee venom with normal and allergic sera. (5) Separation of venom on G-75 Sephadex with analysis of fractions by immunochemical techniques and electrofocusing. (6) Using individual components of venom, determine interactions with immunoglobulin classes in treated (by venom injections) and non-treated allergic individuals.

Major Findings: (1) Mouse LD_{50} assay is a reproducible measure of biological activity of bee venom. (2) Hemolysin assay is a sensitive measure of certain components (not yet defined) of bee venom. (3) Normal serum has a blodking effect on the direct hemolytic activity of venom while serum from treated patients may have an even greater blocking effect. (4) Venom interacts by gel diffusion with non-immune sera of all animals tested yielding a

double precipitin line. (5) Venom has been shown to interact with α, β, and pre-β lipoproteins yielding precipitin lines on gel diffusion.

Significance to Bio-Medical Research and the Program of the Division: (1) Previous investigations of antigens in bee venom with specific antisera have neglected the non-immune interaction which occurs with all sera and lipoproteins. (2) The mouse LD_{50} and hemolysin assay may be useful for establishing biological activity of different preparations of bee venom. (3) Studies of interactions of venom with serum components, immunoglobulins and others, should provide insight into the nature of bee sting sensitivity and effects of immunotherapy (desensitization).

Proposed Course of Project: Continue above studies.

Honors and Awards: None

Publications: None

Serial No. DBS 204
1. Bacterial Products
2. Bacterial Toxins
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Potency Assay of Tetanus Toxoids in Mice

Previous Serial Number: Same

Principal Investigators: M. Carolyn Hardegree, M.D.
Claire Cox, M.S.
Clifford J. Maloney, Ph. D.

Other Investigator: Margaret Pittman, Ph. D., OD

Cooperating Units: Biometrics Section, Office of Director, DBS
Office of Director

Man Years

Total:	0.8
Professional:	0.6
Other:	0.2

Project Description:

Objectives: To evaluate the usefulness of the mouse for the assay of the potency, in International Units, of tetanus toxoids.

Methods Employed: Strains of mice were vaccinated with graded doses of tetanus toxoids and their responses were determined by challenge with tetanus toxin. The unitage of the toxoids was calculated using the International Standards for Tetanus Toxoid, Adsorbed and Plain or using a house reference.

Major Findings: (1) The unitage assigned to lots of tetanus toxoid adsorbed was dependent on the strain of mouse used for assay. (2) The unitage calculated for tetanus toxoids was dependent on the amount of adjuvant present as well as the amount of antigen. (3) The effect of adjuvants on the unitage varied depending on the strain of mouse used for assay. (4) Pertussis vaccine enhanced the protective activity of toxoid in mice.

Significance to Bio-Medical Research and the Program of the Division: The use of International Units (I.U.) for describing the potency of tetanus toxoids provides a quantitative basis for the comparison of toxoids. The I.U. of the toxoid may be related to the duration of the antitoxin response

in man. A mouse assay would be more economical and less space consuming than a guinea pig test if the potency determined in mice could be shown to be relevant to the potency in man. This data should provide information on which strains of mice may be suitable for the assay.

Proposed Course of Project: (1) To examine the effect of different forms of aluminum adjuvant on unitage ($AlPO_4$ vs. Alum). (2) To relate unitage of toxoids obtained in different strains of mice to the unitage obtained in guinea pigs. (3) To establish a reference toxoid suitable for assaying commercial toxoids. (4) To attempt to define factors responsible for the response of different strains of mice to tetanus toxoid.

Honors and Awards: None

Publications: None

Serial No. DBS 13
1. Bacterial Products
2. Bacterial Toxins
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: A Study of the Antigenic Substances of Clostridium tetani

Previous Serial Number: Same

Principal Investigators: M. Carolyn Hardegree, M.D.
Claire Cox, M.S.

Other Investigators: None

Cooperating Units: None

Man Years

Total:	1.0
Professional:	0.7
Other:	0.3

Project Description:

Objectives: (1) To determine and separate the antigens present in culture filtrates of Cl. tetani. (2) To characterize the in vivo and in vitro activities of these substances. (3) To determine their relationship to optimal anti-tetanus immunization.

Methods Employed: Column chromatography, zone electrophoresis, gel diffusion, immunoelectrophoresis, enzyme assays, plasma hemoglobin determination, thin layer chromatography, gas chromatography, animal bioassay, animal immunization and serotonin assays.

Major Findings: Tetanolysin causes the disruption of lysosomes with the release of enzymes and of platelets with the release of enzymes and serotonin.

Significance to Bio-Medical Research and the Program of the Division: It is expected that the investigation will contribute to an understanding of the role of antibodies against Cl. tetani antigens other than the neurotoxin. These antibodies may be necessary for the prophylaxis and the treatment of tetanus. Findings will provide fundamental information for the control of tetanus toxoid and tetanus immune serum globulin.

Proposed Course of Project: Studies will be continued on (1) the isolation and recovery of components of the culture filtrates, (2) the continued characterization of the *in vivo* and *in vitro* effects of the hemolysin, (3) the nature of the inactivation of the hemolysin by ganglioside and ganglioside complexes, and (4) on the relationship of the peptidase to the neurotoxin. Studies will be continued to evaluate the effects of components of culture filtrates on enzymes and vasoactive substances *in vivo*. Findings will be prepared for publication.

Honors and Awards: None

Publications: None

Serial No. DBS 205
1. Bacterial Products
2. Bacterial Toxins
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: A Study of Biologics as Interferon Inducers

Previous Serial Number: Same

Principal Investigators: M. Carolyn Hardegree, M.D.
Stanley Singer, M.D.

Other Investigator: Peter J. Gerone, Sc. D.

Cooperating Units: Laboratory of Pathology
Virus and Rickettsia Division, Ft. Detrick

Man Years

Total:	0.4
Professional:	0.2
Other:	0.2

Project Description:

Objectives: (1) To evaluate the capacity of bacterial vaccines or allergenic extracts of house dust to induce interferon. (2) To evaluate the capacity of these agents to protect mice against respiratory infections.

Methods Employed: (1) Injection of mice by various routes with bacterial vaccines or allergenic extracts of house dust. (2) Determination of interferon activity in pooled mouse sera. (3) Determination of antiviral activity in mice challenged with viruses after treatment with biologics.

Major Findings: (1) A single intravenous injection of allergenic extract of house dust, but not bacterial vaccine, decreased the virus titers in lungs of mice given an aerosol challenge of parainfluenza virus, type 1, but not against a challenge of influenza virus (A_2/Taiwan/64). (2) Multiple, but not single, subcutaneous injection of extract of house dust decreased the rate of infectivity of mice challenged with Sendai virus. (3) No interferon was detected in mice given multiple doses of extract of house dust in contrast to those given a single injection.

Significance to Bio-Medical Research and the Program of the Division: Information has been obtained which shows that certain licensed biologics have the capacity to induce interferon in mice and to protect mice against respiratory infections. This activity may provide a tool for examining the potency of some of these products and may be associated with the clinical usefulness of these types of products.

Proposed Course of Project: (1) To attempt to relate the capacity of these products to induce interferon to their capacity to protect against a viral challenge. (2) To evaluate the capacity of bacterial vaccines to protect against a challenge of bacteria. (3) To relate these findings to a study to be conducted in man (Contract No. NIH-DBS-72-2088) to evaluate the incidence of respiratory infections in recipients of a bacterial vaccine. (4) Findings have been prepared for publication.

Honors and Awards: None

Publications:

Singer, S. H. and Hardegree, M. C.: Induction of Interferon by Bacterial Vaccines and Allergenic Extracts. J. Allergy 47:332-340, 1971.

Serial No. DBS 15
1. Bacterial Products
2. Bacterial Toxins
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Investigation of Toxicity of Oil Emulsified Antigens

Previous Serial Number: Same

Principal Investigators: M. Carolyn Hardegree, M.D.
Michael F. Barile, Ph. D.

Other Investigators: None

Cooperating Unit: None

Man Years

Total:	0.3
Professional:	0.2
Other:	0.1

Project Description:

Objectives: To determine factors responsible for sterile abscesses in recipients of oil emulsified biologics. To examine the use of cell cultures for the evaluation of oil adjuvant component toxicity and to examine the mechanisms of this toxicity.

Methods Employed: Cell cultures, including preparations of mouse macrophages and human lymphocytes, were inoculated with various oils and emulsifiers and the cell toxicity was measured. The effect of various diluents or additives on the toxicity of the oils and emulsifiers was determined.

Major Findings: DMSO enhanced the toxicity of Arlacel A and fatty acids in vivo and in vitro. Poly-L-Ornithine enhanced the toxicity of Arlacel A in vitro. Preliminary data suggests that Arlacel A is toxic for macrophages and lymphocytes as well as for other types of cell monolayers.

Significance to Bio-Medical Research and the Program of the Division: These studies provide information on the reactivity of components of oil emulsified antigens and may be useful in developing specifications for oil emulsified products should they be approved for human use.

Proposed Course of Project: To continue to examine the effect of adjuvant components on cells involved in the immune response and to examine the effect of antigens on the toxicity of oil adjuvant components in cell culture.

Honors and Awards: None

Publications: None

Serial No. DBS 301
1. Bacterial Products
2. Bacterial Toxins
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Evaluation of Reactivity of Diphtheria Toxoids

Previous Serial Number: None

Principal Investigators: M. Carolyn Hardegree, M.D.
Claire B. Cox, M.S.

Other Investigators: None

Cooperating Units: None

Man Years

Total:	0.6
Professional:	0.4
Other:	0.2

Project Description:

Objectives: (1) To evaluate the capacity of diphtheria toxoids to revert to active toxin and to sensitize to beef antigen. (2) To evaluate the intradermal reactivity of adsorbed toxoids.

Methods Employed: Specific toxicity and intradermal reactivity is being examined in guinea pigs after intradermal injection. The capacity of toxoid to sensitize to beef antigen is being evaluated by active and/or passive cutaneous anaphylaxis in guinea pigs.

Major Findings: Active toxin has not been detected in any toxoid products examined.

Significance to Bio-Medical Research and the Program of the Division: It is expected that information will be obtained which will be applicable to the control testing of diphtheria toxoids. Information on the stability of the toxoids and freedom from extraneous antigens is applicable to the safety of the product.

Proposed Course of Project: Studies will be continued to (1) examine the stability of additional toxoids, and (2) to examine toxoids for the presence of beef antigens.

Honors and Awards: None

Publications: None

Serial No. DBS 22
1. Bacterial Products
2. Bacterial Vaccines
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Studies on Cholera and Cholera Vaccines

Previous Serial Number: Same

Principal Investigators: Robert S. Northrup, M.D. (after Nov. 1, 1971)
John C. Feeley, Ph. D. (left NIH Oct. 30, 1971)

Other Investigators: Francis V. Chisari, M.D. (LP)
Ann Sutton
Meade A. Doyle
Lincoln Chen, M.D. (NIAMD)

Cooperating Units: NIAID Cholera Advisory Committee
U.S.-Japan Cooperative Medical Science Program
Laboratory of Pathology
Johns Hopkins University

Man Years

Total:	3.5
Professional:	3.2
Other:	.3

Project Description:

Objectives: Examination of the immunological mechanisms in cholera and the role of toxin, toxoid, and somatic antigen in the development of protective immunity.

Methods Employed: Various toxins and toxoids supplied by the NIH Cholera Advisory Committee were utilized. One lot of fluid toxoid was utilized to prepare experimental aluminum hydroxide or aluminum phosphate adsorbed products. These materials were injected into mice, rabbits, guinea pigs, dogs, or monkeys, or used to challenge exteriorized intestinal loops in chronic monkey surgical preparations. Local reactions were observed, and immune responses were measured in serum and intestinal fluid by hemagglutination and skin permeability assay (antitoxin) and vibriocidal assay (antibacterial antibody). Sera from toxoid-immunized human volunteers were also assayed by hemagglutination. Monkey intestinal loops, dogs, and mice were also challenged with toxin or live V. cholerae, to determine protective responses to previous procedures. Intradermal skin testing and lymphocyte incorporation of

radio-labeled thymidine or leucine were utilized to investigate cellular immune mechanisms. Modifications of standard techniques were used to determine in vitro antibiotic sensitivities of V. cholerae strains. The use of radio-labeled and fluorescein-labeled cholera toxin was explored for antibody assays and studies of toxin localization. Standard histological techniques were used for examination of biopsy specimens.

Major Findings: Subproject 1: Cholera toxoid studies. A purified cholera toxoid preparation inactivated with formalin was found to produce reactions in humans, monkeys, and rabbits which were delayed in onset. This toxoid was shown to revert in vitro to active toxin. During this year histologic studies of reactions in humans following intradermal injection were completed, and suggested that the reaction was initially caused by reversion of the toxoidto toxin, while in a later accentuated phase appeared to result from a combination of toxic and immunologic components. Studies in monkeys showed that the reaction could be prevented by preincubation of the toxoid with monospecific horse antitoxin prior to injection, or by passive intravenous immunization of the monkey with the antiserum. This demonstration that the delayed reaction could be prevented specifically with antitoxin showed conclusively that the observed reactions had been caused by reversion of toxoid to toxin, and confirmed the conclusions from the clinical and histological human studies.

The demonstration that reversion presented a problem with cholera toxoid led to the requirement that future investigational toxoids be shown to be free of reversion. Accordingly, cholera toxoids furnished by contractors and collaborators of the NIH Cholera Advisory Committee were examined for reversion in monkeys. Toxoids prepared by heat inactivation or by separation of natural toxoid (choleragenoid) produced an immediate local reaction after subcutaneous injection because of residual toxic activity, and also reverted, producing a delayed reaction. Glutaraldehyde-inactivated toxoid showed no residual toxin and did not revert. Rabbits and monkeys showed a substantial rise in antitoxin titer in the serum after glutaraldehyde toxoid injections, thus demonstrating the immunogenicity of the products. A large pilot lot of glutaraldehyde inactivated toxoid is being prepared on the basis of this information, and should be available for animal and human testing soon.

The somatic antigen content of cholera toxoids was assessed in order to ensure that protection produced by the toxoids was not due to their somatic antigen component. Mouse protection tests were shown to be insufficiently sensitive to detect the small amounts of somatic antigen in the purified toxoids. The vibriocidal response of rabbits to the toxoids was able to demonstrate differences among them. Column chromatographically prepared toxins and toxoids appeared to be purer than aluminum hydroxide purified toxoids. Future toxoids will be assessed by this technique.

The use of other endotoxin containing bacterial vaccines - pertussis and typhoid - as adjuvants for the formalin toxoid was assessed. Substantial differences in antitoxin levels in rabbits were not induced by this technique, but the possibility of adjuvant activity from reverted toxoid made final conclusion difficult. These studies will be repeated when the non-reverting

glutaraldehyde toxoid becomes available.

As was the case when aluminum hydroxide adsorbed toxoid prepared entirely in this laboratory was employed (see last year's report), no adjuvant effect on antitoxin formation was observed in rabbits immunized with this adsorbed version of the commercially prepared product. Fluid toxoid was employed as a control. The commercially prepared toxoid could only be partially adsorbed on aluminum phosphate in concentrations up to 1 mg of aluminum per ml. Immunization of rabbits with this partially adsorbed product also showed no adjuvant effect on antitoxin formation.

Antibody production in dogs immunized parenterally with toxin and toxoid was determined, and dogs were challenged orally with live organisms at various intervals to determine protective efficacy of the immunizations. The duration of protection produced by toxin in Freund's adjuvant was greater than that seen with liquid toxoid, but protection could be restored to susceptible dogs with orally administered toxoid. The role of intestinal immunity, and the efficacy of oral toxoid administration in this model is currently under further study (work carried out at Johns Hopkins University, Dr. Nathaniel Pierce).

The relative efficiency of 7S and 19S serum immunoglobulin classes in toxin neutralization was assessed using serum fractions prepared by sucrose density gradient ultracentrifugation and by Sephadex column chromatography. In one high-titered dog serum pool 7S fractions appeared to be at least 4 times more efficient in toxin neutralization (PF test) than 19S fractions, although this difference was not as striking in hemagglutination assay. The use of fluorescein and iodine labeled toxin and antitoxin for determination of toxin binding by specific immunoglobulin classes is currently being explored.

In studies with human volunteer sera, the hemagglutination method for antitoxin titration showed a good overall correlation with antitoxin levels determined in other laboratories by in vivo tests (e.g. skin permeability factor). A number of individual sera, however, showed aberrant reactions compared to in vivo tests. A similar situation has been observed by others with hemagglutination tests for diphtheria and tetanus antitoxin.

Subproject 2: Purified somatic antigens: A partially purified cell-free somatic antigen vaccine prepared in Japan was tested in mice for relative protective ability compared to a similar American preparation previously tested in the field, and to reference whole-cell vaccine. The two cell-free preparations showed similar protective ability, but required large quantities of material relative to the whole-cell preparation. Reactivity data in man from Japan suggest that the product is not innocuous, producing local reactions at dose levels necessary for adequate vibriocidal antibody stimulation. At present this product does not appear to offer any significant advantage over whole-cell cholera vaccines.

Subproject 3: Effect of cholera toxin on the immune response: The potent adenyl-cyclase stimulating effect of cholera toxin may produce enhancement of various responses in some tissues, and diminution in others. Last year, preliminary studies in mice showed that toxin could markedly enhance the

appearance of antibody-forming cells in the spleen following immunization with sheep red blood cells. Studies currently in progress have demonstrated that intravenous toxin produces necrosis of spleen red pulp cells and of thymus cortex. The toxin also produces an early rise in splenic adenyl cyclase, with subsequent falloff, but essentially eliminated thymic adenyl cyclase by 12 hours. Studies to correlate these processes with the immune response are in progress.

In contrast to its effect on antibody forming mechanisms, cholera toxin appears to depress the expression of cellular immunity. Preliminary results to date suggest that the toxin depresses both baseline and PHA-stimulated H^3 thymidine incorporation into lymphocytes without depressing cell viability.

Subproject 4: Sensitivity of El Tor and Classical V. cholerae strains to sulfa and trimethoprim. In vitro sensitivity studies demonstrated that Classical V. cholerae strains were substantially more resistant to sulfamethoxazole than were El Tor strains, while both were sensitive to trimethoprim and were synergistically sensitive to a combination of the agents. These studies provide preliminary evidence for the usefulness of sulfa sensitivity testing as a means of differentiating between El Tor and Classical strains.

Subproject 5: Response of monkeys to intra-intestinal V. cholerae toxin or organisms. Thiry-Vella loops were prepared in monkeys and repeatedly challenged with cholera culture filtrate. The loops initially produced diarrheal fluid, later became resistant. This resistance appeared to correlate with the development of antitoxin in loop washings and/or serum.

Other monkeys were placed on low-protein diets for 8-12 weeks, then challenged orally with living V. cholerae. Two of 5 starved monkeys died while 0 of 3 control animals died.

Significance to Bio-Medical Research and the Program of the Division: Cholera represents a major health problem in the world and will doubtless spread to the Americas in the near future. In addition, because of its pathological mechanism cholera provides an ideal model for studies of the interrelationships between local and systemic immunity, antitoxic and antibacterial immunity, and cellular and humoral immunity. Such studies are designed to further our understanding of the mechanisms of resistance to bacterial infection. With this understanding the development of more effective bacterial vaccines and their successful control can be brought about. In particular, cholera toxoid will hopefully provide a significant improvement over existing whole-cell cholera vaccines. The development of suitable adjuvanting procedures is essential for providing maximal duration and extent of protection with the toxoid.

Proposed Course of the Project: To follow through as indicated above.

Honors and Awards: None

Publications:

Northrup, R. S., and Chisari, F. V. Response of monkeys to immunization with cholera toxoid, toxin, and vaccine: Reversion of toxicity of cholera toxoid. J. Infect. Dis. (In press)

Northrup, R. S., Doyle, M. A., and Feeley, J. C. Effect of trimethoprim and sulfamethoxazole in in vitro sensitivity studies of El Tor and Classical Vibrio cholerae strains. Antibiot. Chemother. (In press)

Northrup, R. S., and Chisari, F. V. Cholera toxoid in human volunteers: Clinical and histological studies. Proceedings of the 12th International Congress of Microbiological Standardization, Annecy, France, 1971. (In press)

Serial No. DBS 116
1. Bacterial Products
2. Bacterial Vaccines
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Studies on Typhoid Vaccine

Previous Serial Number: Same

Principal Investigator: K. H. Wong, Ph. D.

Other Investigators: Mary Michael Forlines
John C. Feeley, Ph. D. (left NIH as of November 1, 1971)
Robert S. Northrup, M.D. (replaced Dr. Feeley)

Cooperating Units: None

Man Years

Total:	0.7
Professional:	0.4
Other:	0.3

Project Description:

Objectives: (1) Establishment of a valid potency test for typhoid vaccines which will accurately reflect their efficacy for man. (2) Examination of immunological significance of Vi antigen from Salmonella typhosa in typhoid fever. (3) Elucidation of immune response to typhoid antigen at the cellular level.

Methods Employed: Active and passive mouse protection tests, toxicity assays in normal and actinomycin D-treated mice, enzymatic degradation, physico-chemical fractionation, electroimmunodiffusion, fluorescent antibody, double diffusion in gel, and other immunological and serological techniques.

Major Findings: Subproject 1: Studies on phenol-heat killed and acetone-inactivated typhoid vaccines. Using a Vi-degrading enzyme isolated from Bacillus sphericus, it was found that the non-Vi factor(s) alone in these vaccines were able to specifically protect immunized mice against infection of Salmonella typhosa. The Vi antigen, however, was the major protective factor in an assay where intraperitoneal vaccination and challenge were used.

Although there was no difference in their non-Vi protective activity, the Vi antigen content of the more potent acetone-inactivated vaccine was

greater than that of the phenol-heat killed vaccine and was also of greater protective ability. Treatment of the vaccine with acetone seemed to "bond" the Vi antigen to the cell wall, thus increasing its immunogenicity.

Besides the difference in Vi antigen, the acetone-inactivated vaccine was less toxic than the phenol-heat killed vaccine. In actinomycin D-treated mice, LD_{50} for the acetone-inactivated vaccine was 4.5×10^6 bacteria as compared with an LD_{50} of 1.4×10^6 bacteria for the phenol-heat killed vaccine.

Subproject 2: Isolation and quantitation of Vi antigen. Vi antigen was purified from a saline extract of Citrobacter strain 5396/38 by enzymic digestion, concentration with ethanol, and precipitation with hexadecyl trimethylammonium bromide. Vi antigen prepared by this method was at least 10 and 200 times, respectively, more protective in mice than two other preparations isolated by an earlier method utilizing acid hydrolysis.

A sensitive and specific method of electroimmunodiffusion was developed for quantitation of Vi antigen. As little as 10 µg per ml could be measured in a crude cell extract. It is likely that it will offer a convenient way for comparing quantitatively extractable Vi antigen from different lots of typhoid vaccine prepared by the same method.

A modification of the purification method, using hexadecyltrimethylammonium bromide, is under study for isolation of Vi antigen from Salmonella typhosa Ty 2.

Significance for Bio-Medical Research and the Program of the Division: Although the presence of Vi antigen is correlated with virulence and immunogenic properties of Salmonella typhosa in mice, its significance in human immunity remains to be ascertained and its influence in potency assay of typhoid vaccines in mice has been of much concern to biologics control laboratories. Understanding of the influence of the various protective factors is essential in establishing a potency test which will accurately reflect the prophylactic efficacy of the vaccine in man.

Field trials have demonstrated that acetone-inactivated vaccine is superior to phenol-heat killed vaccine. Unfortunately, adverse reactions to both vaccines due to endotoxin and other toxic substance in the bacterial cells are quite pronounced. Improvement of vaccines may be achieved by separation of toxic components from protective factors.

Proposed Course of Project: Further work is aimed at (1) solving the long-existing controversy of whether Vi antigen is necessary for protection in man; (2) elucidating immune response to the typhoid bacillus and its purified antigens at cellular level.

Honors and Awards: None

Publications:

Wong, K. H., Feeley, John C., and Pittman, M.: Effect of a Vi-degrading enzyme on potency of typhoid vaccines in mice. J. Infect. Dis. (In press)

Serial No. DBS 136
1. Bacterial Products
2. Bacterial Vaccines
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Evaluation of Microbially Controlled Animals in Control Testing and Infection

Previous Serial Number: Same

Principal Investigator: Carl E. Miller, D.V.M.

Other Investigators: Kwei-Hay Wong, Ph. D.
John C. Feeley, Ph. D.
Mary Michael Forlines

Cooperating Units: None

Man Years

Total:	0.8
Professional:	0.6
Other:	0.2

Project Description: Part I. Interplay of Intestinal Microflora on Mouse Susceptibility to Endotoxin. Project discontinued.

Part II. Role of the Indigenous Microflora in Resistance of Mice to Infection with Vibrio cholerae. Project discontinued.

Part III. Epidemiological Significance of Chronic Carriers of Rough Vibrio cholerae.

Objectives: To determine if chronic carriers of rough Vibrio cholerae could be a source of cholera from season to season.

Methods Employed: Rough forms of V. cholerae isolated from convalescent carriers were passed serially in gnotobiotic, bacteria-free mice. Selection of smooth forms of cholera vibrios was facilitated by treatment with guinea pig complement and anti-rough serum. The completely smooth isolates were tested for fluid production in infant rabbits and for capillary permeability in rabbit skin.

Major Findings: Smooth forms of V. cholerae, not unlike the original field isolates were recovered after the third passage in mice. The fluid production in infant rabbits and capillary permeability in rabbit skin of

the new isolates was markedly increased over the original culture from the human carrier.

Significance to Bio-Medical Research and Program of the Division: Cholera in humans is usually seasonal in the endemic areas. The location of V. cholerae in the off-season is not known. Demonstration of revitalization of smooth cholera vibrios from chronic carriers of rough vibrios may provide a partial answer to this question.

Proposed Course of Project: No further study is anticipated. Results will be published.

Honors and Awards: None

Publications:

Miller, Carl E., and Feeley, John C.: Competitive effects of intestinal microflora on Vibrio cholerae in gnotobiotic mice. *In* Proceedings of the 6th Joint Conference of U.S.-Japan Cooperative Medical Science Program Symposium on Infection and Immunity of Cholera. Ed.: Hideo Fukumi and M. Ohashi, National Institute of Health, Tokyo. 1971.

Serial No. DBS 182
1. Bacterial Products
2. Bacterial Vaccines
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Standardization of Pseudomonas Vaccine

Previous Serial Number: Same

Principal Investigators: John C. Feeley, Ph. D.
Edward B. Seligmann, Jr., Ph. D.

Other Investigators: Jane F. Farber (LCA)
Otis L. Green (LCA)
Kwei-Hay Wong, Ph. D.
Carl E. Miller, D.V.M.

Cooperating Units: Laboratory of Control Activities

Man Years

Total:	0
Professional:	0
Other:	0

Project Description: No work was carried out on this project during the current Fiscal Year.

Serial No. DBS 207
1. Bacterial Products
2. Bacterial Vaccines
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1971

Project Title: Bioassay of Endotoxin in Bacterial Vaccines

Previous Serial Number: Same

Principal Investigator: Kwei-Hay Wong, Ph. D.

Other Investigators: John C. Feeley, Ph. D. (left NIH November 1, 1971)
Robert S. Northrup, M.D. (replaced Dr. Feeley)
Carl E. Miller, D.V.M.

Cooperating Units: None

Man Years

Total:	0.1
Professional:	0.1
Other:	0.0

Project Description:

Objectives: To study the feasibility of the assay system established in the previous year for quantitation of endotoxin in bacterial vaccines.

Methods Employed: Actinomycin D was used to potentiate endotoxicity in mice. LD_{50} values were calculated from death-survival curve. Results were compared with those from the mouse weight gain test.

Major Findings: (1) Endotoxin content of different lots of typhoid, pertussis, and pseudomonas vaccines was determined and referenced to an endotoxin preparation from E. coli. Results obtained by titration with actinomycin D-treated mice were comparable to those from the mouse weight gain test. Assay with actinomycin D, however, appeared to be more sensitive and specific. (2) Mice of the Balb/c strain were more susceptible to the lethal effect of endotoxin than the NIH strain. This difference in susceptibility appeared to be genetic as gnotobiotic mice of these two strains also manifested the same order of difference. As expected, gnotobiotic mice were more resistant to endotoxin than conventional mice.

Significance for Bio-Medical Research and the Program of the Division: This assay system with actinomycin D-treated mice provides a sensitive and apparently specific method for titration of endotoxin in bacterial products. In view of the genetic difference among mouse strains in susceptibility to endotoxin, it seems feasible to produce mouse strains which are either more susceptible or resistant to endotoxin than those conventionally used.

Honors and Awards: None

Publications: None

Serial No. DBS 302
1. Bacterial Products
2. Bacterial Vaccines
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Immune responses of laboratory animals to meningococcal polysaccharide vaccines

Previous Serial Number: None

Principal Investigator: K. H. Wong, Ph. D.

Other Investigators: Mary Michael Forlines
John C. Feeley, Ph. D. (left NIH as of November 1, 1971)
Robert S. Northrup, M. D. (replaced Dr. Feeley)

Cooperating Units: None

Man Years

Total:	0.7
Professional:	0.4
Other:	0.3

Project Description:

Objectives: To explore the possibility of establishing a suitable animal assay system for meningococcal polysaccharide vaccines.

Methods Employed: Active protection tests were done with animals including mice, guinea pigs, and hamsters. For detection of antibody, the following techniques were used: hemagglutination, bactericidal, indirect fluorescent antibody, and plaque formation by spleen cells on agar.

Major Findings: Subproject 1: Animal protection tests. Groups of animals were immunized intraperitoneally or subcutaneously with varying doses of purified polysaccharide vaccine from meningococcus group C provided by the Walter Reed Army Institute of Research. They were challenged 14 days later i.p. with a culture of meningococcus group C suspended in 5% mucin. No protection was observed.

Subproject 2: Antibody response in laboratory animals. Mice, guinea pigs, and hamsters were vaccinated i.p. or s.c. with varying doses of the antigen and bled 7 days after the last injection. No antibody response has been demonstrated by hemagglutination and bactericidal assays. Other assay methods are being planned.

(42)

Significance for Bio-Medical Research and the Program of the Division: Efficacy of meningococcal polysaccharide vaccine (group C) has been demonstrated in several field trials. However, this antigen appeared to be non-immunogenic in the animals tested. In the absence of a suitable animal assay system, standardization of these antigens would have to rely on their physico-chemical properties. It remains to be seen if immunogenicity can be assured by the use of physico-chemical measurements.

Proposed Course of Project: The use of a carrier or adjuvant is being considered. More sensitive techniques will be used for detection of antibody and other animals including monkeys will be included in this study.

Honors and Awards: None

Publications: None

Serial No. DBS 101
1. Bacterial Products
2. Mycobacterial and Fungal Antigens
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Studies on Tuberculosis Vaccines

Previous Serial Number: Same

Principal Investigator: Sotiros D. Chaparas, Ph. D.

Other Investigator: Sally R. Hedrick

Cooperating Units: None

Man Years

Total:	0.4
Professional:	0.1
Other:	0.3

Project Description:

Objectives: To compare various BCG vaccines for antigenic similarities, suitability in inducing delayed hypersensitivity, and as adjuvants.

Methods Employed: Culture filtrates from 12 strains of BCG vaccine were prepared and compared by immunoelectrophoretic analyses with a standard antigen-antibody reference system for M. tuberculosis. Precipitin bands produced were then identified with those of the reference system. Guinea pigs, sensitized with each of the 12 strains of BCG, were skin tested with homologous and heterologous tuberculins.

Major Findings: All 12 strains of BCG shared most of the identifiable antigens of the reference system. No significant differences in reactivity of the tuberculins in homologously or heterologously sensitized guinea pigs could be observed.

Significance for Bio-Medical Research and the Program of the Division: The remarkable feature of these studies is that so many antigenic similarities were found between 12 strains of BCG that have been maintained over numerous generations under varying conditions in different laboratories throughout the world. These studies are of value in evaluating and characterizing licensed BCG vaccines and other vaccines used over the world.

Proposed Course of Project: To test the ability of different strains of BCG to induce cellular immunity effective in cancer cell rejection.

Honors and Awards: None

Publications:

Chaparas, S. D., Wera, T., and Hedrick, S. R.: Antigens in culture filtrates of 12 BCG strains compared by electrophoresis, gel diffusion and skin tests. International symposium on BCG vaccine. Symp. Series Immunobiol. Standard. 17:133-136 (1971). Previously reported as "In Press")

Serial No. DBS 103
1. Bacterial Products
2. Mycobacterial and Fungal Antigens
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Hypersensitivity and Specificity of Fungal Diagnostic Substances for Dermal Tests

Previous Serial Number: Same

Principal Investigators: Sotiros D. Chaparas, Ph. D.
Edward B. Seligmann, Jr., Ph. D. (LCA)
Vernon Fuller, Ph. D. (LCA)

Other Investigators: None

Cooperating Unit: Laboratory of Control Activities

Man Years

Total:	0.3
Professional:	0.3
Other:	0.0

Project Description:

Objectives: (1) To develop suitable laboratory procedures for use in potency and identity tests for Fungal Diagnostic Substances for Dermal Tests. (2) To prepare standards for commonly used skin test reagents.

Methods Employed: Antigens from spherule and mycelial growth of several strains of Coccidioides immitis prepared on contracts have been analyzed by acrylamide electrophoresis and homologous and heterologous skin tests.

Major Findings: Three of six strains have been investigated thus far, and acrylamide patterns and cross skin reactions suggest a great deal of antigenic similarity.

Significance to Bio-Medical Research and the Program of the Division: (1) The apparent similarities of strains of Coccidioides immitis may mean that the type or number of strains used to prepare coccidioidin for skin testing may not be very important. (2) These results will be helpful in determining and characterizing materials to be used as a suitable standard preparation for Coccidioidin. This will permit the Division to better control commercial preparations for uniformity and consistency of production.

Proposed Course of Project: Three more strains will be studied and evaluated with the 3 previous strains. With information gathered in DBS laboratories and on contract, a new standard for Coccidioidin will be prepared and evaluated.

Honors and Awards: None

Publications:

Chaparas, S. C., Fuller, V. J., and Seligmann, E. B.: Laboratory Procedures for potency testing of blastomycin, histoplasmin and Coccidioidin. Proc. Soc. Exp. Biol. Med. (In press)

Serial No. DBS 135
1. Bacterial Products
2. Mycobacterial & Fungal Antigens
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Mycobacterial Antigens: Immunological Specificity and Antigenic Analysis

Previous Serial Number: Same

Principal Investigator: Sotiros D. Chaparas, Ph. D.

Other Investigators: Sally R. Hedrick
Edward B. Seligmann, Jr., Ph. D. (LCA)
James T. Dove, M.D., (CDC)
Gerard Wijsmuller, M.D., (CDC)
Mary Long (CDC)

Cooperating Units: Laboratory of Control Activities
Tuberculosis Branch, Center for Disease Control
Antigen Subcommittee - Tuberculosis Program
U.S.-Japan Cooperative Medical Program, GMB, NIAID

Man Years

Total:	1.3
Professional:	0.6
Other:	0.7

Project Description: This project is divided into V parts.

Objectives: The overall objectives are: (1) to compare skin reactive specificity of tuberculins in groups of animals sensitized with homologous and heterologous species of Mycobacterium; (2) to characterize antigens from different species of mycobacteria and to determine skin reactivity and specificity of isolated fractions; (3) to investigate possible mechanisms of isoniazid-associated hepatitis; (4) to investigate with in vitro techniques, mechanisms of delayed sensitivity.

Part I. Preparation of sensitizing organisms and skin test antigens for Mycobacterium intracellulare (Battey) to be used as reference materials in the control of Battey antigens for Dermal testing (with E. B. Seligmann).

Methods Employed: Five hundred Blake bottles were inoculated with M. intracellulare. Four hundred were harvested early for organisms to be

used in sensitization and the other 100 were permitted to lyse for source material to study antigens of this species.

Major Findings: One thousand one hundred thirty-five gms of organisms and 8.3 gms of antigens have been produced.

Part II. Studies on sharing of antigens from various mycobacterial species (in collaboration with Antigens Subcommittee of Tuberculosis Panel - U.S.-Japan Cooperative Medical Program).

Methods Employed: Antigens from 12 species of mycobacteria were compared to a reference Mycobacterium tuberculosis antigen-antiserum by immunoelectrophoresis.

Major Findings: A great deal of antigenic sharing among the various mycobacteria was observed. Representative organisms from the 4 Runyon Groups were employed and each organism produced antigens in common with M. tuberculosis. In fact, of 11 identifiable antigens in the reference system, none could be considered unique for M. tuberculosis.

Part III. In vivo and in vitro reactivity of fractions prepared from BCG culture filtrates.

Methods Employed: An additional in vitro method is being explored which measures the amount of early protein synthesis by the incorporation of C^{14}-leucine after antigenic stimulation of lymphocytes.

Major Findings: The measurement of early protein synthesis in lymphocytes after antigenic stimulation in general correlated well with the in vitro lymphocyte transformation test.

Part IV. Studies of the reactivity of PPD tuberculins in guinea pigs sensitized with various species of mycobacteria (with G. Wijsmuller and Mary Long).

Methods Employed: Guinea pigs were sensitized with heat-killed organisms of various mycobacterial species suspended in water-in-oil emulsions. Tuberculins with or without Tween 80 were obtained from the CDC. The tuberculins included Parke-Davis PPD lot T5504, Connaught PPD lot 68, PPD-S lot 49608 and PPD-606I.

Major Findings: Profiles of reactivity of each tuberculin in groups of guinea pigs sensitized with different mycobacterial species were very similar.

Part V. Cells from patients with Isoniazide (INH) associated hepatitis were studied by the lymphocyte transformation test in order to determine responsiveness to this drug (with James T. Dove).

Methods Employed: Lymphocytes from peripheral blood of patients with INH-associated hepatitis were exposed in vitro to INH using the lymphocyte transformation procedure.

Major Findings: Lymphocytes of patients with INH-associated hepatitis did not become transformed when exposed to INH. Reactivity to PPD and PHA was normal.

Significance to Bio-Medical Research and the Program of the Division: These studies lend insight into some of the difficulties experienced in preparing highly specific skin test antigens. The fact that antigens truly unique for M. tuberculosis have not yet been detected accounts for the great deal of cross reactivity of tuberculins tested in people infected with various mycobacteria. Unique antigens may be present, however, in antigens from other mycobacterial species. It was encouraging to be able to show that the various PPD's were very similar qualitatively with respect to reactivity in guinea pigs sensitized with various mycobacteria reactivity. Thus, although different strains of M. tuberculosis or M. bovis may be used in tuberculin production, reactivity of these products in populations infected with different mycobacterial species may be expected to be similar. The in vitro correlates of delayed hypersensitivity are useful in studying mechanisms of reactivity as well as aids in determining differences in reactivity of tuberculins and fractions. Lymphocytes of patients with INH-associated hepatitis failed to become transformed in vitro. This observation does not lend support to an immunological basis of the disease.

Proposed Course of Project: Some emphasis will be placed on the testing of the Battey organisms for suitability in the induction of sensitivity to the proposed standard Battey antigen. Such a preparation may then be distributed to manufacturers for sensitizing guinea pigs to determine lot to lot consistency of standardized batches of Battey antigen. It is also hoped to characterize and study unheated culture filtrates from Battey organisms for the purpose of detecting and isolating antigens unique for Battey, or at least antigens that do not cross-react with M. tuberculosis antigens. Further applications of in vitro procedures for delayed sensitivity are planned.

Honors and Awards: None

Publications:

Chaparas, S. D., Thor, D. C., and Hedrick, S. R.: Comparison of the lymphocyte transformation, inhibition of macrophage migration and skin tests using dialyzable and nondialyzable tuberculin fractions from Mycobacterium bovis (BCG). J. Immunol. 107:149-153, 1971. (Previously reported as "Accepted for publication")

Dove, J. T., Chaparas, S. D., and Hedrick, S. R.: Failure to demonstrate transformation of lymphocytes of patients with isoniazid-associated hepatitis. Amer. Rev. Resp. Dis. (In Press)

Chaparas, S. D., and Bowser, R.: The effects of route of antigen administration on the induction of delayed skin reactivity and antibody in the rat. Res. Comm. in Chem. Path. and Pharmacol. (In Press)

Janicki, B. W., Chaparas, S. D., Daniel, T. M., Kubica, G. P., Wright, G. L., Jr., and Yee, G. S.: A reference system for antigens of Mycobacterium tuberculosis. Amer. Rev. Resp. Dis. 104:602-604, 1971.

Janicki, B. W., Chaparas, S. D., Daniel, T. M., Kubica, G. P., Wright, G. L., Jr., and Yee, G. S.: Mycobacterial Antigen Workshop: An evaluation of a reference system for antigens of M. tuberculosis. Amer. Rev. Resp. Dis. (In Press)

Serial No. DBS 17
1. Bacterial Products
2. Mycoplasma
3. Bethesda, Md

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Mycoplasma in Human Disease and Biologics

Previous Serial Number: Same

Principal Investigator: Michael F. Barile, Ph. D.

Other Investigators: Ruth L. Kirschstein, M.D. (LP)
Stanley H. Singer, M. D. (LP)
Joseph G. Tully, Ph. D. (NIAID)
Richard A. DelGiudice (HRC)
Theodore R. Carski, M. D. (HRC)
Jerome Kern, Ph. D. (FL)
Ruth M. Lemcke, Ph. D. (LI)
Donald B. Riggs
Marion W. Grabowski

Cooperating Units: Laboratory of Pathology, DBS
Laboratory of Microbiology, NIAID
Huntingdon Research Center, Inc., Baltimore
Flow Laboratories, Inc., Rockville
Lister Institute of Preventive Medicine, London, England

Man Years

Total:	2.9
Professional:	0.9
Other:	2.0

Project Description:

Objectives: (1) To characterize the biological, biochemical and serological properties of Mycoplasma. (2) To develop new methods or improve existing methods for primary isolation and detection of Mycoplasma as they pertain to the test for Mycoplasma required for certain biologic products. (3) To investigate the role of Mycoplasma in diseases of man and animals.

Methods Employed: (1) Culture procedures were used, including several different broth, semi-solid agar-broth and agar media containing various supplements, and cultures were incubated aerobically and in a carbon dioxide-nitrogen atmosphere. (2) Mycoplasma species were identified on the basis of their serological, metabolic, pathogenic and membrane protein acrylamide

electrophoretic mobility properties. (3) Several cell cultures were infected with either mycoplasmas and/or viruses and determinations were made on the effect of mycoplasma on interferon production and on virus yields.

Major Findings: (1) A new simian Mycoplasma species, designated M. primatum, was characterized on the basis of its biological, biochemical and serological properties and cell membrane protein electrophoretic patterns. The 18 strains isolated from oral and urogenital tissue of the African green monkey, Cercopithecus aethiops, and one strain isolated from man were related to each other and unrealted to the 40 well-established species of Mycoplasma and Acholeplasma. (2) Mycoplasmas were isolated from 121 of 424 commercial bovine serum lots examined. Of 70 strains examined for identity, 44 were identified as M. arginini, 15 as A. laidlawii, 8 were unclassified strains of Acholeplasma sp., and 3 were unclassified strains of Mycoplasma sp., and may represent a new species. Data indicate that M. arginini and A. laidlawii are common contaminants of commercial bovine sera and of cell cultures, and that bovine serum is a major source of bovine mycoplasma contamination of cell cultures. (3) Mycoplasma arginini Barile et al., 1968, and Mycoplasma leonis Heyward et al., 1969 were found to be synonyms on the basis of comparisons of the original species descriptions and of the serological and biochemical characteristics of authentic strains, including the type strain, of each of the organisms. According to the rules of nomenclature, the correct name of the species formed by the union of these two taxa is M. arginini Barile et al., which name has priority over M. leonis Heyward et al. (4) Fourteen conjunctival strains of mycoplasma were isolated from infected sheep and goats in two separate naturally occurring outbreaks of keratoconjunctivitis (pink eye). The biological and serological properties and cell protein patterns of these strains indicated that these conjunctival strains were related to each other and were unrelated to the 40 recognized Mycoplasma and Acholeplasma species and serotypes. We are proposing that this group of mycoplasmas be named M. conjunctivae. The role of M. conjunctivae in the etiology of keratoconjunctivitis in sheep and goats remains to be determined. (5) Continuing studies on mycoplasma-virus-cell culture interactions using primary and continuous cell cultures derived from mouse tissues indicate that chronic mycoplasma infection of cell cultures can result in either an increased or a decreased virus yield, and can influence the size and morphology of the virus plaque. The virus titer and the size and morphology of the plaque is influenced by the strain and species of Mycoplasma and virus as well as the type of cell used in the interaction. (6) Mycoplasmas isolated by 2 U.S. licensed manufacturers from 2 lots of rubella vaccine and from 3 lots of vaccine for veterinarian use were identified as bovine species of mycoplasmas; 2 were related to the unclassified but distinct bovine group 11 (M165/69) of Leach, and 3 were related to Acholeplasma laidlawii. These vaccines were destroyed by the manufacturers.

Significance to Bio-Medical Research and the Program of the Division: These studies may provide information on the pathogenicity of mycoplasmas for man and animals. The improved culture procedures developed are applicable to the isolation and detection of mycoplasmas in biologics which are subject to mycoplasma contamination risk.

Proposed Course of Project: To pursue studies to improve methods for primary isolation of mycoplasmas from biologics and from tissues associated with disease. To consider assay methods for requirements for Mycoplasma pneumoniae vaccine products.

Honors and Awards: None

Publications:

DelGiudice, R. A., Carski, T. R., Barile, M. F., Lemcke, R. M., and Tully, J. G.: Proposal for classifying human strain Navel and related simian mycoplasmas as Mycoplasma primatum sp. n. J. Bacteriol. 108: 439-445, 1971.

Barile, M. F., and Kern, J.: Isolation of Mycoplasma arginini from commercial bovine sera and its implication in contaminated cell cultures. Proc. Soc. Exp. Biol. Med. 138:432-437, 1971.

Tully, J. G., DelGiudice, R. A. and Barile, M. F.: Synonymy of Mycoplasma arginini and Mycoplasma leonis. Internat. J. System. Bacteriol. 22:47-49, 1972.

Barile, M. F., DelGiudice, R. A., and Tully, J. G.: Isolation and characterization of Mycoplasma conjunctivae sp. n. from sheep and goats with keratoconjunctivitis. Infection and Immunity. 5:70-76, 1972.

Singer, S. H., Ford, M., Barile, M. F., and Kirschstein, R. L.: Effect of mycoplasmas on virus replication and plaque formation in mouse cells. Proc. Soc. Exp. Biol. Med. 139:56-58, 1972.

Barile, M. F.: Normal flora of simians. J. Infect. Dis. (In press).

Barile, M. F., and DelGiudice, R. A.: Isolation of mycoplasmas and their rapid identification by plate epi-immunofluorescence. *In* Pathogenic Mycoplasmas, The Ciba Symposium, 1972 (In press).

Serial No. DBS 104
1. Bacterial Products
2. Office of the Chief
3. Bethesda, MD

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Studies on Pertussis Vaccine

Previous Serial Number: Same

Principal Investigators: Charles R. Manclark, Ph. D.
Clifford J. Maloney, Ph. D. (OD)
Fred S. Yamada (DCRT)
Phillip P. McGrath (LP)
Francis V. Chisari, M.D. (LP)

Other Investigators: James F. Marshall
Ethan P. Summers

Cooperating Units: Pharmaceutical Manufacturers' Association
Division of Computer REsearch and Technology

Man Years

Total:	3.4
Professional:	1.3
Other:	2.1

Project Description:

Objectives: The overall objective is that the pertussis vaccine component of products issued by U.S. licensed establishments has optimum properties, adequate and stabile potency through the dating period and relative freedom from untoward reactivity. Objectives of current studies are (1) to study and monitor the laboratory assayed potency and freedom from toxicity of commercial vaccines submitted for release; and (2) to fractionate and characterize the biologically and immunologically active components of pertussis vaccine.

Methods Employed: Subproject 1: Mouse potency tests to determine adequacy and stability of potency of pertussis vaccine. Mouse weight gain tests to determine freedom from toxicity of pertussis vaccine. The results of all official pertussis potency tests done by the Laboratory of Bacterial Products for the last six years are being used for a computer study to evaluate the control potency testing of pertussis vaccine.

Measurement of pertussis vaccine toxicity is done by a determination of the early and late weight changes in mice, the lethal dose in mice, histamine sensitizing activity, necrotizing activity in the skin of suckling mice, ability to induce edema in the foot pad of the rat, rabbit pyrogenicity, ability to produce lymphocytosis/leukocytosis in the mouse, etc.

Subproject 2: Bordetella pertussis cells have been fractionated by a variety of physical, chemical, and enzymatic procedures. The isolated fractions are characterized for their content of mouse protective antigen, lymphocytosis promoting factor, dermonecrotic toxin, histamine sensitizing factor, hemolysin, endotoxin, and serospecific antigens. Electron microscopy studies are being used to determine if specific function can be related to subcellular organelles or macromolecules.

Major Findings: Subproject 1: No significant changes in potency and toxicity of pertussis vaccine submitted for release were noted.

The measurement of rat footpad edema at 24 hours permits the differentiation of vaccines containing dermonecrotic toxin. In the mouse weight gain and ascites tests, the late weight gain (viz. reduced rate of weight gain) appears to be related to heat labile toxin(s). The abaility to produce ascites (mouse sensitization test) does not appear to be a pharmacologic (toxic) reaction or related to the presence of heat labile toxin(s). These tests appear to measure the presence of a variety of biologically active materials present in varying quantities and combinations and may have their greatest value in serving as an assay system in the isolation and characterization of each of the measured components so that eventually a more simple, specific, less expensive, more rapid serological procedure can be evolved and used for assessing the specific components of pertussis vaccine which are responsible for its reactogenicity.

Many workers have proposed that an assay for histamine sensitizing factor could be used as a rapid assay for protective antigen in pertussis vaccine. Studies completed in this laboratory and others have shown that the administration of graded doses of unadsorbed experimental pertussis vaccines would give a graded response in mice and permit the calculation of an HSD_{50}. Diphtheria, Tetanus and Pertussis adsorbed vaccines from each of nine manufacturers were used to sensitize mice so that an HSD_{50} could be determined for each respective vaccine and this related to the potency of the vaccine. Less than half of the vaccines tested gave graded responses and HSD_{50} estimates within reasonable limits. It is concluded that the determination of an HSD_{50} as an estimate of potency of commercial, multiple antigen products such as Diphtheria, Tetanus, and Pertussis Vaccine, adsorbed is of little or no value.

These same commercial vaccines were assayed for their lymphocytosis promoting factor (LPF) content and it was shown that a graded response was obtained when graded doses were administered mice and that these results were reproducible and permitted a vaccine to be characterized by its LPF content.

Subproject 2: Proteins isolated from B. pertussis cells demonstrated a strong tendency to aggregate upon extraction. Evidence has been generated which suggests that these aggregations are the result of hydrophobic interactions between the various protein components. Recent work with non-ionic detergents, EDTA, and enzymes has permitted better extraction of the cells and greater stability of the extracted proteins than previously experienced.

A necessary prerequisite to a clinical study of pertussis immunity and the role of pertussis vaccine as the cause of untoward reactivity in children, including the rare neurological reactions, febrile reactions, and local inflammation and pain at the injection site, would be the extraction and characterization of suitable antigens for use in sero-epidemiological studies. Several prospective antigens have been prepared and sent to the CDC for a laboratory serological evaluation.

Attempts to relate protective activity to subcellular structure are in a preliminary stage. Studies with lymphocytosis promoting factor would indicate that LPF is soluble and about 25,000-50,000 molecular weight. The cytological events which occur following the injection of dermonecrotic toxin-containing whole cell preparation of B. pertussis into the skin of suckling mice have been studied and will serve as a basis for studies to be done with purified dermonecrotic toxin to elucidate its mode of action.

Significance to Bio-Medical Research and the Program of the Division: Subproject 1: The projects are related directly to the control of the potency and safety of pertussis vaccine and the relation of laboratory assayed toxicity to untoward reactivity in children.

Subproject 2: Experimental studies are directed at elucidating the cause of adverse reactions, including the rare neurological reactions, febrile reactions, and local inflammation and pain at the injection site, which follow the administration of pertussis vaccine and the isolation and characterization of the pertussis protective antigen(s).

Proposed Course of Project: Subproject 1: (1) To continue the testing and surveillance of potency and safety of pertussis vaccine. (2) To complete the computer review of pertussis potency testing, the method of recording and evaluating test results, and an examination and reassessment of current Regulations for pertussis vaccine.

Subproject 2: (1) To isolate and characterize the protective antigen of B. pertussis as a necessary first step towards the improvement of pertussis vaccine. (2) To initiate studies to determine the actual incidence and nature of clinical reactions to the administration of pertussis vaccine. (3) To initiate research to determine genetic and physiologic factors involved in the growth of B. pertussis that affect the potency and toxicity of pertussis vaccine.

Honors and Awards: None

Publications:

Murata, R., Perkins, F. T., Pittman, M., Scheibel, I., and Sladky, K. International Collaborative Studies on the Pertussis Vaccine Potency Assay. Part Played by the Challenge in the Mouse-Protection Test. Bull. Wld. Hlth. Org., 44:673-687, 1971.

Serial No. DBS 138
1. Bacterial Products
2. Office of the Chief
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Selective Breeding to Establish a "Standard" Mouse

Previous Serial Number: Same

Principal Investigators: Charles R. Manclark, Ph. D.
Carl T. Hansen, Ph. D. (DRS)

Other Investigators: James F. Marshall
Ethan P. Summers
William J. Hinkle (DRS)

Cooperating Unit: Division of Research Services

Man Years

Total:	0.6
Professional:	0.2
Other:	0.4

Project Description:

Objectives: To establish a mouse strain that has superior and consistent characters for the potency and toxicity assays of biologics, particularly bacterial vaccines.

Methods Employed: Thirty-seven breeding pairs of a "sensitive" line and twenty-two pairs of a "resistant" line have been selected and bred. The progeny of each are being tested for their ability to be sensitized to histamine by pertussis vaccine. Mice with the highest ability as well as those with the highest resistance are being selected for further breeding.

Major Findings: (1) The colony is now in its twelfth generation with a few of the breeding pairs in the ninth, tenth, or eleventh generation. (2) The evidence is clearcut that both sensitivity and resistance to histamine sensitization by pertussis vaccine is genetically determined. (3) When sensitized with one opacity unit of pertussis vaccine and challenged with 100 mg of histamine diphosphate per kg of body weight, selectively bred animals of the sensitive line now manifest a 70% sensitivity compared to the 31% of the starting colony. Mice of the resistant colony demonstrate only a 3-4% sensitivity. (4) The variability of sensitivity has decreased from a standard deviation of $\pm$ 20% in the original colony to $\pm$ 15% in the present

sensitive line. (5) HSFS mice have been shown to be sensitive to anaphylactic shock induced by antigen-antibody complexes, whereas, HSFR mice are resistant to anaphylactic shock with the same complexes.

Significance to Bio-Medical Research and the Program of the Division: The availability of a "standard" mouse for potency and toxicity assays of pertussis vaccine and other bacterial products should decrease variability in tests and give greater assurance of the validity of the estimate of potency and toxicity of the products.

Proposed Course of Project: To pursue basic immunogenetic studies directed toward the establishment of an isogenic strain of mouse which carries the genetic marker(s) for histamine sensitivity and reactivity to pertussis protective antigen(s). (2) To study the nature and inheritance of the genetic markers which determine the ability of a mouse to be sensitized to histamine. (3) To determine if the difference is quantitative, mice will be selectively bred to raise or lower the threshold of activity in HSFS and HSFR mice, respectively. (4) To study the feasibility of using F_1 hybrids of two different HSFS strains to retain the "sensitive" character of inbred strains and yet obtain the advantages of hybrid vigor resulting from such a first generation cross. (5) The data collected to this point are being analyzed by automatic data processing methods.

Honors and Awards: None

Publications: None

CONTRACT NARRATIVES
LABORATORY OF BACTERIAL PRODUCTS
DIVISION OF BIOLOGICS STANDARDS
July 1, 1971 through June 30, 1972

Allergenic Products Section

UNIVERSITY OF CALIFORNIA MEDICAL SCHOOL (PH-43-68-632)

Title: Determination of Potency and Efficacy of the Active Ingredient of Poison Oak Extract by Clinical Studies

Contractor's Project Director: William B. Epstein, M.D.

Project Officer (DBS): Harold Baer, Ph. D.

Assistant Project Officer (DBS): Mitchell H. Friedlaender, M.D.

Objectives: To determine whether a pure preparation of the active principle of poison oak can hyposensitize individuals sensitive to poison oak.

Methods Employed: The active principle was isolated from fresh leaves under Contract (PH-43-64-76) at Columbia University and was prepared for clinical use by the Pharmaceutical Development Service of the NIH. The contractor administers this material to sensitive patients orally and topically. Skin tests prior to and during treatment are used to assess efficacy. Blood and urine samples are taken at regular intervals for laboratory examination to aid in determining whether the treatment produces any toxic effects.

Major Findings: The study shows that it is possible to reduce the sensitivity of sensitive individuals.

Significance to DBS and Bio-Medical Research: Poison ivy and oak extracts are subject to license, but it has not been established that they do, in fact, hyposensitize sensitive individuals. Since it now appears that it is possible to reduce sensitivity, this will enable standards to be set for such extracts.

Proposed Course of Project: No further work is projected on this Contract. A new contract is envisioned which will provide expanded studies to establish dosage schedules and duration of efficacy.

Date Contract Initiated: January 15, 1968

Current Annual Level: $22,501

CONTRACT NARRATIVES
LABORATORY OF BACTERIAL PRODUCTS
DIVISION OF BIOLOGICS STANDARDS
July 1, 1971 through June 30, 1972

Allergenic Products Section

COLUMBIA UNIVERSITY (PH-43-64-76)

Title: Biochemical Studies on Poison Ivy, Poison Oak and Sumac

Contractor's Project Director: Prof. Charles R. Dawson

Project Officer (DBS): Harold Baer, Ph. D.

Assistant Project Officer (DBS): Mitchell H. Friedlaender, M.D.

Objectives: (1) To isolate the active principles (urushiols) of poison ivy and oak and to supply these to the Project Officer for laboratory and clinical studies. (2) To isolate the individual components of poison ivy urushiol for laboratory and clinical studies. (3) To isolate the urushiol of poison oak, to determine the complete chemical structure of the components and to supply quantities of these pure components to the Project Officer. (4) To synthesize various substances related to pentadecylcatechol to permit studies of sensitization and tolerance by the Project Officer.

Methods Employed: Various isolation, synthetic, and analytical methods are employed.

Major Findings: (1) Quantities of pure urushiols have been prepared for laboratory and clinical studies. (2) Quantities of the four components of poison ivy urushiol have been prepared and sent to DBS. (3) The structure of some of the components of poison oak urushiol has been determined. Although this study is not complete, it is now known that poison oak urushiol is different from poison ivy urushiol. (4) Compounds closely related to pentadecylcatechol have been prepared that are less toxic than pentadecylcatechol, are poor sensitizers but potent toleragens.

Significance to DBS and Bio-Medical Research: This project will provide information on a group of licensed products, Poison Ivy Extract, Poison Oak Extract, and, eventually, Poison Sumac Extract, and related products that are potentially subject to license, i.e., Cashew Nut Oil Extract. The synthetic substances being examined for the relationship of chemical structure to ability of catechols to sensitize may provide a less reactive substitute for the naturally occurring catechols for diagnosis or treatment of poison ivy, oad, or sumac sensitivity. As a result of this work, it should be possible to develop a potency assay for poison oak and ivy extracts that can be applied to products manufactured under license.

Proposed Course of Project: To continue until objectives are reached.

Date Contract Initiated: November 18, 1963

Current Annual Level: $33,887

CONTRACT NARRATIVES
LABORATORY OF BACTERIAL PRODUCTS
DIVISION OF BIOLOGICS STANDARDS
July 1, 1971 through June 30, 1972

Allergenic Products Section

OHIO STATE UNIVERSITY (NIH-69-65)

Title: Identification, Isolation, and Culture of Mites from House Dust

Contractor's Project Director: G. W. Wharton, Ph. D.

Project Officer (DBS): Harold Baer, Ph. D.

Assistant Project Officer (DBS): E. B. Seligmann, Jr., Ph. D.

Objectives: (1) To examine house dust samples from various parts of the United States for their content of mites and other insects. (2) To determine the genus and species of the mites. (3) To isolate each species of mite, to produce pure cultures of each, and grow gram quantities of each. (4) To study the nutritional and physical requirements for optimal growth of each species.

Methods Employed: (1) Various support substances such as dust linters and nutrients such as dog food are used to propagate mites. (2) Investigate the influence of environmental and nutritional factors on mite growth in order to permit large scale production of several species of mites, especially those of the genus Dermatophagoides. (3) Determine whether it is possible to produce axenic cultures of mites or cultures grown in media low in antigenicity.

Major Findings: (1) House dust samples collected from widely different areas of the U.S. (many of which were supplied to the contractor by DBS) were shown to contain four species of mites of the genus Dermatophagoides. The most common species was D. farinae and has been called the North American house dust mite. This material has been published in Science. (2) Several different culture techniques have been studied and found to be useful in producing gram quantities of mites; the ingredients have been supplied to DBS by the contractor.

Significance to DBS and Bio-Medical Research: Although it now appears that mites contribute to the allergenicity of house dust, they do not provide the sole or even major house dust allergen. Nevertheless, now that they have been discovered, various manufacturers of allergenic extracts are considering the need to manufacture extracts of these animals. This contract has provided useful information in establishing that house dust contains only 2 species of the genus Dermatophagoides, methods for their culture have been worked out, and differences in the cultural and physiological characteristics determined.

Proposed Course of Project: No further work seems necessary at this time and the contract will not be renewed.

Date Contract Initiated: November 26, 1968

Current Annual Level: $20,000

CONTRACT NARRATIVES
LABORATORY OF BACTERIAL PRODUCTS
DIVISION OF BIOLOGICS STANDARDS
July 1, 1971 through June 30, 1972

Allergenic Products Section

MAYO FOUNDATION (NIH-DBS-72-2087)

Title: Measurement of the Potency of Allergenic Extracts by the Radioallergosorbent Test (RAST)

Contractor's Project Director: Gerald J. Gleich, M.D.

Project Officer (DBS): Harold Baer, Ph. D.

Objectives: To measure the potency of allergenic extracts by the RAST and to compare the activity with tests in sensitive humans.

Methods Employed: (1) Extracts will be coupled to cellulose and its reactivity measured with serum of allergic humans. (2) The ability of extracts to inhibit the reactivity of a standard extract-polymer will be measured with a serum from a reactive human.

Major Findings: The project has just started, therefore there are no findings as yet.

Significance to DBS and Bio-Medical Research: This project should enable the Division to set up a laboratory test that will correlate the reactivity of allergenic extracts with reactivity in allergic humans. This would enable the Division to set potency standards for products for which there are currently no standards.

Proposed Course of Project: To continue until the objectives are reached.

Date Contract Initiated: April 1, 1972

Current Annual Level: $24,320

CONTRACT NARRATIVES
LABORATORY OF BACTERIAL PRODUCTS
DIVISION OF BIOLOGICS STANDARDS
July 1, 1971 through June 30, 1972

Bacterial Toxins Section

BIONETICS RESEARCH LABORATORIES (PH-43-66-1164 and PH-43-67-1483)

Title: Oncogenic Screening of Biological Products in Hamsters

Contractor's Project Director: John Landon, Ph. D.

Project Officer (DBS): Ruth Kirschstein, M.D.

Assistant Project Officers (DBS): Claire B. Cox, M.S.
Stanley Singer, M.D.

Objectives: A three-year study designed to test viral vaccines (live and killed), rickettsial and bacterial vaccines and toxoids for possible oncogenic effects in hamsters was done. The study has been completed.

Major Findings: None of the biological products that were evaluated appeared to be oncogenic for newborn hamsters following a single subcutaneous injection.

Significance to DBS and Bio-Medical Research: Information has been obtained pertaining to the safety and purity of biological products with respect to their oncogenic potentials as measured in the hamster system.

Proposed Course of Project: This contract terminated June 1969.

Date Contract Initiated: June 30, 1966.

Current Annual Level: None

Publications:

Cox, C. B., Landon, J., Valerio, M. G., Palmer, A., Kirschstein, R. L. and Singer, S. H. Lack of significant oncogenicity of biological products in hamsters. Appl. Microbiol. 1972 (In press)

CONTRACT NARRATIVES
LABORATORY OF BACTERIAL PRODUCTS
DIVISION OF BIOLOGICS STANDARDS
July 1, 1971 through June 30, 1972

Bacterial Toxins Section

MIDWEST RESEARCH INSTITUTE (PH-43-67-679)

Title: An Objective Appraisal of the Safety of the Aluminum and Oil Adjuvants with Regard to their Potential for Oncogenic Action

Contractor's Project Director: John O. MacFarlane, Ph. D.

Project Officer (DBS): Carolyn Hardegree, M.D.

Assistant Project Officer (DBS): Pinya Cohen, Ph. D.

Objectives: To evaluate the oncogenic potential of aluminum and oil adjuvants.

Methods Employed: Groups of inbred and random bred male and female mice were injected subcutaneously with a variety of antigens, antigens adsorbed to aluminum adjuvants, aluminum compounds, and components of oil adjuvants. Other groups received similar treatment plus a known chemical carcinogen. Gross and microscopic examinations of the mice will be made over a period of 90 weeks for the direct studies and a period of 52 weeks for the co-carcinogen studies to determine the development and/or enhancement of tumors.

Major Findings: The observation period for this phase is incomplete and therefore no results are available for either the direct or co-carcinogenic studies.

Significance to DBS and Bio-Medical Research: Evaluation of adjuvant preparations in animals will provide data on the safety of these materials. The results of these studies will help provide a basis for decisions on the use and potential safety of oil adjuvants.

Proposed Course of Project: Animals in the co-carcinogen study will be sacrificed at 52 weeks. Observation will continue for animals on direct carcinogen study. Gross and histopathologic examinations will be made of all animals dying or sacrificed. Statistical analyses of data will be made. Funds have been allocated through March 31, 1973, and the contract is expected to continue into 1974.

Date Contract Initiated: February 24, 1967

Current Annual Level: $229,930

Publications:

Murray, R., Cohen, P., Hardegree, M. C. Mineral Oil Adjuvants: Biological and Chemical Studies. Annals of Allergy 30:146-15, 1972.

CONTRACT NARRATIVES
LABORATORY OF BACTERIAL PRODUCTS
DIVISION OF BIOLOGICS STANDARDS
July 1, 1971 through June 30, 1972

Mycobacterial and Fungal Antigens Section

UNIVERSITY OF CALIFORNIA, DAVIS (NIH 71-2082)

Title: Studies on Coccidioidin Derived from Single Strains of *Coccidioides immitis*

Contractor's Project Director: Demosthenes Pappagianis, M.D., Ph. D.

Project Officer (DBS): Sotiros D. Chaparas, Ph. D.

Assistant Project Officer (DBS): Edward B. Seligmann, Jr., Ph. D.

Objectives: To produce coccidioidins from the mycelial phase of 6 strains of *Coccidioides immitis*, to characterize these products, to determine their reactivity and to prepare materials as potential standards.

Methods Employed: Organisms were grown in synthetic medium for varying periods of time, and the filtrates harvested. Skin reactivity and strain specificity are determined in sensitized animals and human beings. Studies of the stability of activity of the coccidioidins are being performed at temperatures ranging from -15°C to 50°C in the presence and absence of Tween 80.

Major Findings: Freeze-dried mycelial preparations for sensitization of guinea pigs and coccidioidin for each of the 6 strains have been produced. Some variation in growth rates and quantity of product for each strain have been noted. Qualitatively, the products appear to be similar.

Significance to DBS and Bio-Medical Research: A new standard for coccidioidin may be prepared from these materials which will be well characterized with regard to potency in man, role of strains used for production, stability of reactivity, and chemical analyses.

Proposed Course of Project: To investigate if growth in shaking cultures has any value over growth in stationary cultures in producing coccidioidin. To complete testing in human beings and animals. To characterize the coccidioidins chemically and immunologically. To present the Division with at least 10 gm of coccidioidin from each strain.

Date Contract Initiated: March 30, 1971

Current Annual Level: $26,500

Publication: None

CONTRACT NARRATIVES
LABORATORY OF BECTERIAL PRODUCTS
DIVISION OF BIOLOGICS STANDARDS
July 1, 1971 through June 30, 1972

Mycobacterial and Fungal Antigens Section

UNIVERSITY OF CALIFORNIA, BERKELEY (NIH-71-2083)

Title: Spherule Coccidioidin in the Dectection of Coccidiomycosis

Contractor's Project Director: Hillel B. Levine, Ph. D.

Project Officer (DBS): Sotiros D. Chaparas, Ph. D.

Assistant Project Officer (DBS): Edward B. Seligmann, Jr., Ph. D.

Objectives: To prepare coccidioidin from spherules of six strains of Coccidioides immitis and to characterize these substances and determine their reactivities.

Methods Employed: Organisms were made to produce spherules in Converse medium and then the spherules were lysed. Studies to determine stability of activity of spherule coccidioidin (spherulin) with and without Tween 80 have been performed at various temperatures. Infected guinea pigs and mice were tested with different spherulins and the reactivities compared. Protein, carbohydrate and nitrogen determinations were made.

Major Findings: Material prepared with strains Silvera and 46 were of comparable reactivity, but the Woodville strain yielded poorly-active spherulin. Qualitatively, the 3 strains appeared to be similar. There was a suggestion that some reactivity may be lost on freeze-drying. Coccidioidin prepared from spherules (Spherulin) was about 10 times more reactive on a weight basis than coccidioidin prepared from mycelial form of growth.

Significance to DBS and Bio-Medical Research: Spherule coccidioidin is a more potent skin test reagent than mycelial coccidioidin, and may serve as a more suitable reference or standard than mycelial coccidioidin, but further work is required. The results reported here point up a similar antigenic makeup of spherulins from different strains but yields may be low for some strains. Thus, only good spherulin producers should be considered as sources of reference material. This information will assist the Division in selecting the most suitable materials to serve as references or standards.

Proposed Course of Project: To prepare and characterize spherulins from the remaining 3 strains. To compare skin reactivity and specificity of all 6 strains in homologously and heterologously sensitized animals. To provide 10 gm of spherulin to the Division for possible use as a reference.

Date Contract Initiated: April 5, 1971

Current Annual Level: $26,039

Publications: None

CONTRACT NARRATIVES
LABORATORY OF BACTERIAL PRODUCTS
DIVISION OF BIOLOGICS STANDARDS
July 1, 1971 through June 30, 1972

Allergenic Products Section

JOHNS HOPKINS UNIVERSITY (NIH-69-2010)

Title: Development of Information on the Activity of Allergenic Extracts

Contractor's Project Director: Philip Norman, M.D.

Project Officer (DBS): Harold Baer, Ph. D.

Assistant Project Officer, (DBS): Mitchell H. Friedlaender, M.D.

Objectives: To determine the human reactivity of allergenic extracts of grass, ragweed pollens, and mite and house dust extracts.

Methods Employed: (1) Skin tests were carried out with extracts in sensitive humans to determine the minimum reacting doses. (2) A determination was made of the amount of each extract required to cause the release of 50% of the histamine from leucocytes of sensitive individuals. Grass pollen and mite extracts will be fractionated to isolate the active allergens.

Major Findings: Studies were carried out with extracts of 6 different grass pollens manufactured by 5 different companies. The findings were analogous to those previously found for ragweed pollen extracts. There was a high correlation between human reactivity and Group I antigen content, but no correlation with PNU content. Continued studies on mites failed to demonstrate a specific mite allergen and tended to confirm the finding that all house dust allergy is not mite allergy.

Significance to DBS and Bio-Medical Research: The correlation of the human potency with a laboratory potency assay for allergenic extracts will make it possible for the first time to prescribe a potency assay for an allergenic extract. This will also help to determine whether current dating periods are suitable.

Proposed Course of Project: To continue until the objectives are attained.

Date Contract Initiated: March 1969.

Current Annual Level: $24,892

Annual Report of the
Laboratory of Blood and Blood Products
Division of Biologics Standards
July 1, 1971 through June 30, 1972

During the past year, the unprecedented interest in blood banking shown by Congress, by the Department, and by the general public has had a significant impact on the staff of the Laboratory of Blood and Blood Products. It has also required considerable staff time in handling requests for information from many quarters. Many of these requests have been directed to Dr. Ashworth, who has just completed his first full year as Laboratory Chief. Nevertheless, he has been able to become involved in all aspects of the diversified regulatory and scientific responsibilities of the Laboratory.

The number of Sections in the Laboratory was increased to five. This resulted from a redistribution of the staff and responsibilities of the former Blood and Blood Derivatives Section in accordance with the products regulated and the nature of scientific investigations performed. The Laboratory now consists of the new Blood Bank Products, Plasma Derivatives, and Immunology Sections plus the previously existing Diagnostic Reagents and Coagulation Sections.

I. Intramural Research Activities: The research interests of the Laboratory included projects in every section, each one devoted to inquiries designed to give information to support the regulatory responsibilities of the Division.

Work has continued on studies of the stability of the product of plasma fractionation, i.e., albumin, immune globulins and coagulation components. In the latter case, an intense interest in assay methods has been given major attention. The lack of precision in available methodology often results in difficulty in reaching agreement with manufacturers on potency. Investigations on the various diagnostic reagents ranged from long range research on the nature of certain phenomena observed in products to projects of more immediate importance to product purity or potency. The various projects are geared to the knowledge, experience, and capabilities of staff so that each can participate in the research program of the Laboratory. The loss of two positions and the impact of personnel ceilings have made it very difficult to devote more than token staff time to research interests. A strict rationing of available resources and assignment of priorities has been necessary in order to accomplish required regulatory work and still permit individual investigative projects.

II. Other Than Research Activities: The number of products for which the Laboratory is responsible and the number of personnel available for attention to these products results in a one person-one product situation with little chance or time for back-up or team work situations. There are 72 different licensed blood products and 27 staff members. Nevertheless, the Laboratory has been able to handle each of its areas of responsibilities by establishing objectives that do not result in emphasis of certain areas to the exclusion of others.

This year has seen the almost universal adoption by blood banks of the test for Hepatitis Associated (Australia) Antigen which became licensed at the end of the preceding year. The publication of the Notice of Rule Making requiring this test in licensed blood banks is expected momentarily. The perfection of this test and the expected improvement of its sensitivity in the coming years holds promise for blood therapy free of the danger of hepatitis.

Fortunately the number of applications assigned to the Laboratory dropped although the Laboratory's share of those coming into the Division remains high. Of the 365 licensing actions received in the Division, 223 were assigned to the Laboratory of Blood and Blood Products. The number pending at the end of the reporting period was significantly less than a year ago. See Table I.

The number of lots submitted to the Laboratory for control testing remained at a level approximately the same as last year. See Table II. The reduction in tests performed was due to two factors, a redefinition of a "test" in the evaluation of blood bank products and a conscious rationing of staff time devoted to this purpose so that other responsible duties would not be neglected.

There are 12 authorized inspectors and 2 inspector designees currently on the Laboratory staff. During the past year 329 separate location inspections were carried out requiring 292 man days of travel. See Table III. As in the past a number of theinspection visits were unscheduled to accommodate changes in locations by existing licensees or new establishments applying for license. The staff maintains flexibility so that the unanticipated inspections are performed expeditiously.

Work continues on various Additional Standards. Proposed Standards for Normal Serum Albumin (Human), Plasma Protein Fraction (Human), Single Donor Plasma (Human) and Source Plasma (Human) have been completed and forwarded for clearance prior to publication as Notices of Proposed Rule Making.

Dr. Finlayson has continued his leadership of meetings planned to allow individuals in the Laboratory to present discussions of their duties and scientific work to the staff of the Laboratory. These meetings have been beneficial in fostering understanding of each person's role and interests and in addition have allowed an opportunity to obtain advice, counsel and criticism from peers.

Miss Crouch devoted almost full time for three months as a member of a Task Group gathering facts for a Department legislative program on blood banking. Her long experience and knowledge of this field was of great value in producing a comprehensive factual review.

STATUS OF LICENSE APPLICATIONS

April 1, 1971 through March 31, 1972

TABLE 1

	ESTABLISHMENT APPLICATIONS	PRODUCT APPLICATIONS	AMENDMENTS	TOTAL
No. Pending 4-1-71	25	106	89	220
No. Received	12	51	160	223
No. Recommended	12	67	173	252
No. Withdrawn or Inactive	7	21	18	46
No Pending 3-31-72	14	69	58	141

LBBP-3

CONTROL TESTING OF BLOOD AND BLOOD PRODUCTS

April 1, 1971 through March 31, 1972

TABLE II

	LOTS TESTED			Total No. Lots	TESTS PERFORMED			Total No. Lots
	Pre-licensing	Inspection	For Release		Purity	Potency	Safety	
Blood Fractions	133	13	2210	2356	1149	1023	1410	3582
Diagnostic Reagents	175	66	1595	1836	8658	4718	0	13376
Whole Blood, Single Donor Plasma, Red Blood Cells, Cryoprecipitated AHF	158	578	4	740	0	81	3252	3333
TOTALS	466	657	3809	4932	9807	5822	4662	20291

LBBP-4

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CONTROL ACTIVITIES REQUIRING TRAVEL STATUS

April 1, 1971 through March 31, 1972

TABLE III

ACTIVITY	LOCATIONS	CONTRACTS	MAN DAYS OF TRAVEL
Inspections	329		292
Support of Contracts		2	3

Serial No. (NIH-DBS) 33
1. Blood & Blood Products
2. Plasma Derivatives
   Coagulation
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Preparation, Characterization, Stability and Potency of Blood Products

Previous Serial Number: Same

Principal Investigators: David L. Aronson, M.D.
Pinya Cohen, Ph.D.
John S. Finlayson, Ph.D.
Andrew M. Young, M.S.

Other Investigators: B. Lynne Armstrong
Mae Moody
Joan Roelands, B.A.
Peter C. Stang, M.S.

Man Years:
Total: 2.6
Professional: 2.1
Other: 0.5

Patient Days: None

Project Description:

Objectives: To obtain more detailed information on the characteristics of blood products, to determine changes in their properties which occur during processing and storage, and to develop improved methods and standards.

Methods Employed: Standard serological, immunological and physicochemical methods have been used. Additional specialized methods which are under development are described in the following section.

Major Findings: a) Comparative studies designed to adapt faster and more efficient electrophoretic methods to the analysis of plasma proteins have been extended to the use of cellulose acetate electrophoresis. Analysis of Normal Serum Albumin (Human), Plasma Protein Fraction (Human) and Immune Globulin (Human) by this method have shown that cellulose acetate electrophoresis, compared to disc and moving boundary electrophoresis, will be the most useful routine procedure for measuring the purity of plasma derivatives sent to this Division for release. (Roelands, Moody and Cohen)

b) A study has been initiated on the effects of repeated heating of stabilized Normal Serum Albumin (Human). This was undertaken to determine physicochemical and immunologic changes in albumin when the preparation is heated to eliminate pyrogens. The results have shown that a protein concentration of 25% placental albumin was the most stable to repeated heating, venous albumin the least stable, and mixtures of venous and placental albumin were of intermediate stability. Albumin (25%) from placental and mixed sources showed slight increases in the amount of dimer compared to monomer. During the initial heating cycles venous albumin showed increased dimerization; however, upon continued heating a reversible dimerization occurred, i.e., the amount of dimer decreased with a concomitant increase in the amount of monomer. With 5% albumin from different sources there were no significant changes in relative concentrations of monomer and dimer. Ultracentrifugal analyses showed slightly more dimer in 25% albumin than 5% albumin solutions; however, the sensitivity of these analyses did not permit precise quantitation of differences. Preliminary immunoelectrophoretic experiments have revealed changes in the character of the precipitin arc obtained with albumin heated repeatedly. Agar diffusion and immunoelectrophoretic experiments have shown that heating destroys the capacity of other proteins in commercial albumin solutions to precipitate in the presence of antibody produced against the product. (Moody, Roelands, Young and Cohen)

c) A study has been undertaken to examine the in vivo and in vitro immunologic relationships of serum albumin, aggregated albumin and albumin microspheres. The in vivo relationships are being tested by sensitizing groups of guinea pigs with one of the albumin preparations, boosting and challenging the guinea pigs with either the same or one of the other two preparations, and observing for anaphylaxis. The results have shown that guinea pigs can be sensitized to albumin microspheres and will undergo anaphylactic shock when they are later challenged with serum albumin, but a booster injection of microspheres following sensitization with microspheres prevents anaphylaxis in guinea pigs challenged with serum albumin. Microspheres may sensitize guinea pigs but when used as the challenging antigen they can not induce anaphylaxis regardless of the albumin antigen used for sensitization. Aggregated albumin is a strong sensitizing and challenging antigen; antigenically it appears more similar to serum albumin than to albumin microspheres. The in vitro serologic studies have shown that albumin microspheres elicit very little precipitating antibody in rabbits. Serum albumin produces a weak precipitin cross-reaction with rabbit antimicrosphere serum. Efforts are being made to find methods to solubilize microspheres so as to evaluate their serologic activity compared to aggregated and serum albumin. (Stang, Roelands and Cohen)

d) As a result of a report in the literature concerning a positive Coombs test for anti-D in a child receiving a large dose of Immune Serum Globulin (Human), the level of anti-D in numerous commercial lots of Immune Serum Globulin (Human) has been analyzed. The level of this antibody varied among lots and manufacturers but was found to be low in most of the lots tested. As a result of this analysis a proposal for maximum levels of

isoagglutinins in immune globulin products has been sent to licensed manufacturers. (Roelands)

e) The half-life of radionuclide-labeled albumin products has prevented the Division from releasing each lot of radiobiologics as is done with other licensed products. In January 1972 a surveillance program of radiobiologics was begun involving five different products produced by four manufacturers. Samples and protocols of each licensed product are submitted quarterly to the Division for testing and review. Manufacturers are notified if any results do not conform to the regulatory standards. (Stang)

f) Partly as a result of studies on the levels of blood group substances A and B in placental immune globulins (see Annual Report 1971), manufacturers of plasma proteins derived from placentas have been asked to identify the placental source of the product in their labeling and call attention to the possible side effects of these compounds. (Cohen)

g) The second phase of the study of the effects of prior heating on stability of immunoglobulin G during storage of human serum (see Annual Report 1971) has been completed. In this phase unfractionated, pooled serum was heated for 36 to 72 hours at 45 °C or 50 °C and stored at temperatures ranging from -22 °C to 37 °C. Unlike the heating of Immune Serum Globulin (Annual Reports 1969 and 1971), heating whole serum failed to diminish fragmentation of immunoglobulin during subsequent storage and actually produced some enhancement of the degradation. (Finlayson and Armstrong)

h) In an attempt to devise a method of predicting the stability of Immune Serum Globulin (Human) during storage, a large number of samples of this product were subjected to a series of analyses. Each sample was initially analyzed in the ultracentrifuge and tested for the presence of plasmin by the fibrin plate method. Sterile portions of each sample were then stored under the following conditions: 1) -23 °C; 2) 5 °C; 3) 24 °C; and 4) 24 °C in the presence of urokinase. Aliquots of materials stored under conditions 2, 3, and 4 were analyzed after storage for 1 year, 9 months, and 4 weeks, respectively. Although the experiments are only partially complete, inasmuch as storage will be continued, the following observations can be made. The degree of breakdown (as indicated by the relative amount of 3.5 S material) after 1 year at 5 °C correlated well with that measured after incubation for 4 weeks at 24 °C in the presence of urokinase (correlation coefficient, 9.79). Furthermore, of the 22 samples which underwent no degradation under the latter condition, none exhibited breakdown after 1 year at 5 °C. On the other hand, storage at 24 °C for 9 months resulted in more breakdown than did incubation at this temperature for 4 weeks in the presence of urokinase. Although there was a general correlation between the extents of degradation under these two conditions, approximately 40 percent of the samples which contained no 3.5 S material after 4 weeks' incubation with urokinase had undergone detectable breakdown after 9 months at 24 °C. (Young and Aronson)

Significance to Bio-Medical Research and the Program of the Division: The Laboratory of Blood and Blood Products is responsible for control of the safety, purity and potency of products derived from blood. Such surveillance involves the possession of knowledge regarding the stability of biological products as well as the availability of appropriate control test methods and technical standards. The project fulfills these needs by providing information leading to more realistic dating periods and control test methods.

Proposed Course of Project: This is a continuing project.

Honors and Awards: None

Publications:

Cohen, P. and Hudson, H. H.: Blood group substances A and B. Levels in placental and venous globulins. Vox Sanguinis 21:311-318, 1971.

Cohen, P., Berkeley, W. H. and Seligmann, Jr., E. B. Coral snake venoms. In vitro relation of neutralizing and precipitating antibodies. Amer. J. Tropical Medicine and Hygiene. 20:646-649, 1971.

Finlayson, J. S., Armstrong, B. L., and Young, A. M.: Reversibility of human immunoglobulin G dimerization. Acta Radiol. Suppl. 310:114-123, 1971.

Lacerte, J. M., Kane, V. M., and Cohen, P.: U. S. licensed blood banks: evaluation of whole blood and its components. Transfusion. In press.

Serial No. (NIH-DBS) 81
1. Blood & Blood Products
2. Coagulation
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: The Clotting and Fibrinolytic Systems

Previous Serial Number: Same

Principal Investigator: David L. Aronson, M.D.

Other Investigators: B. Lynne Armstrong
John S. Finlayson, Ph.D.
Anne J. Mustafa
Rachel Wheeler

Cooperating Units: National Heart and Lung Institute (Dr. John J. Pisano)
Clinical Center (Dr. Harvey R. Gralnick)

Man Years:
Total: 2.5
Professional: 1.1
Other: 1.4

Patient Days: None

Project Description:

Objectives: To purify and characterize components of these systems and to develop an understanding of their chemistry.

Methods Employed: The methods employed ranged from measurements of clotting factors in vitro (by standard assay systems and by those developed in this Laboratory) to fractionation and analytical procedures typical of protein biophysics and biochemistry.

Major Findings: a) Further progress on the purification of human Factor IX has been made. It has been possible to identify, by polyacrylamide gel electrophoresis in the presence of SDS, a protein band associated with the zymogen and a band associated with the active coagulant. These measurements indicate a molecular weight of 65,000 for the former and 50,000 for the latter. No synthetic substrates have been found for IX_a. Early results with a modified test for activated Factor IX are promising. (Aronson, Mustafa and Wheeler)

b) Preliminary results utilizing polyacrylamide gel electrophoresis in SDS and chromatography on DEAE cellulose indicate that activation of Factor X by Factor VII or by Factor IX_a in the presence of Factor VIII yields a similar form of X_a. (Aronson and Mustafa)

c) The bacterial protease "thermolysin" rapidly and apparently quantitatively converts Factor X to X_a. (Aronson and Mustafa)

d) In cooperation with Dr. Michael Mosesson, Downstate Medical Center, the heterogeneity of human plasma fibrinogen manifested as differences in solubility has been investigated. The Bβ and γ subunit chains prepared from fibrinogen fractions of different solubilities were indistinguishable. By contrast, the high-solubility fibrinogens were shown to be catabolic intermediates which had undergone various degrees of degradation in the COOH-terminal portion of the Aα chains. These high-solubility circulating catabolites were found to be quite similar to highly clottable derivative fractions produced by treatment of purified fibrinogen with plasmin in vitro. (Finlayson and Armstrong)

e) In cooperation with Dr. John Pisano, National Heart and Lung Institute, and Dr. Michael Mosesson, the capacity of high-solubility fibrinogen fractions to form ε-(γ-glutamyl)lysine crosslinks in the presence of thrombin and Factor XIII was measured. Under conditions in which "conventional" low-solubility fibrinogen formed 6 moles crosslink/mole fibrin, the high-solubility fibrinogens formed approximately 3. Since polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate showed that crosslinking of the γ chains was complete, and since the Aα chains of the high-solubility fibrinogens have undergone COOH-terminal degradation, it could be concluded that the Aα chains of fibrinogen contain crosslinking sites in the COOH-terminal region. (Finlayson and Armstrong)

f) In cooperation with Dr. Michael Mosesson, the two types of human fibrinogen (designated peak 1 and peak 2) distinguishable by chromatography on DEAE cellulose (see Annual Report 1971) were separated, and their subunit chains were isolated and examined. No differences were found between the Aα chains of the two types of fibrinogen or between their Bβ chains. During chromatography on CM cellulose the γ chains of both types of fibrinogen resolved into two approximately equal populations, designated γ-1 and γ-2. Upon electrophoresis, the γ-1 and γ-2 chains of peak 1 fibrinogen migrated as a single band, whereas those of peak 2 fibrinogen separated into two bands, designated γ and γ'. The γ' chains, which were the more anodal (the γ chains of peak 2 fibrinogen migrated like the corresponding chains of peak 1 fibrinogen) amounted to about half of the γ chain population of peak 2 fibrinogen. All γ chain variants appear to have the same molecular size. The presence of the γ'variant accounts for the charge difference between peak 1 and peak 2 fibrinogen. (Finlayson and Armstrong)

g) Continued cooperation with Dr. Harvey Gralnick, Clinical Center, has led to the recognition and characterization of another abnormal fibrinogen, tentatively designated fibrinogen Bethesda II. This fibrinogen did not differ from normal fibrinogen in electrophoretic mobility, sedimentation coefficient, elution pattern on DEAE cellulose, or hexose and sialic acid contents. It did differ with respect to thrombin and reptilase clotting times (prolonged) and fibrin monomer aggregation (delayed onset; decreased aggregation rate). Unlike fibrinogen Bethesda I (fibrinogen Bethesda, Annual

Report 1971), fibrinopeptide release showed only a minor abnormality in the early phase. (Finlayson and Armstrong)

h) In the course of studying the crosslinking of a third abnormal fibrinogen ("fibrinogen Oklahoma") in collaboration with a guest worker, Robert Morton, a simple but effective precautionary measure was developed for examining whole plasma clots by the polyacrylamide gel-sodium dodecyl sulfate electrophoretic technique. When such clots were solubilized and reduced immediately after the clotting step, crosslinking of the α chains appeared to be minimal. This was shown to be the result of contamination with plasma albumin which, owing to similarity in molecular size, migrates at the same rate as the α chain. The albumin could be removed by washing the clot at 5 °C in 0.154 M NaCl. Employing this step prior to solubilization and reduction led to gel electrophoretic patterns which permitted a more accurate estimate of the extent of fibrin crosslinking. (Finlayson and Armstrong)

Significance to Bio-Medical Research and the Program of the Division: Control of the purity and potency of therapeutic blood derivatives used to treat coagulation and thrombotic disorders involves detailed understanding of the mechanism of the clotting and fibrinolytic processes and the chemistry of the assay methods. This understanding is used in improving the validity and precision of control tests.

Proposed Course of Project: This is a continuing project.

Honors and Awards: None

Publications:

Aronson, D. L., and Mustafa, A. J.: Two forms of the human prothrombin converting enzyme. Proc. Soc. Exptl. Biol. Med. 137:1262-1266, 1971.

Aronson, D. L.: Current status of standardization of Factor IX concentrates. Thromb. Diath. Haemorrh. Suppl. 45:447, 1971.

Gralnick, H. R., Givelber, H. M., Shainoff, J. R., and Finlayson, J. S.: Fibrinogen Bethesda: a congenital dysfibrinogenemia with delayed fibrinopeptide release. J. Clin. Invest. 50:1819-1830, 1971.

Finlayson, J. S., and Morton, R. O.: Gel electrophoresis for assessing fibrin crosslinking: a precaution. Clin. Chim. Acta 36:254-256, 1

Serial No. (NIH-DBS) 82
1. Blood & Blood Products
2. Coagulation
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Proteins in Body Fluids

Previous Serial Number: Same

Principal Investigators: David L. Aronson, M.D.
John S. Finlayson, Ph.D.

Other Investigator: Anne J. Mustafa

Cooperating Units: National Cancer Institute (Dr. Michael Potter)

Man Years:
Total: 0.3
Professional: 0.2
Other: 0.1

Patient Days: None

Project Description:

Objectives: To complement available data on blood proteins with a reservoir of basic information on the properties of and methods suitable for use on the proteins of blood and other fluids, including proteins involved in the clotting and fibrinolytic systems (see projects 33 and 81).

Methods Employed: The fractionation methods are those typically used in protein separations. Analytical methods include physical, chemical and spectrophotometric procedures as well as enzyme assays.

Major Findings: a) The venom of Echis carinata has been found to convert human prothrombin to thrombin with no apparent change in the activities of Factor IX and Factor X. A plasma protein fraction treated with this venom has been found useful to study the interaction of Factors VIII, IX and X. Dr. John Suttie, University of Wisconsin, has found that this venom will also activate the apparently inactive prothrombin-like protein isolated from dicumarol treated cows. (Aronson and Mustafa)

b) In cooperation with Dr. Oliver Smithies, University of Wisconsin, and Dr. Michael Potter, National Cancer Institute, studies have been undertaken to determine partial amino acid sequences of the purified components of the major urinary protein complex of normal inbred mice. Availability of sequence information should permit comparison not only with similar components from mice with different genetic characteristics but also with

other, biologically active proteins. Experiments to date, utilizing the Edman degradation procedure, have confirmed conclusions drawn from tryptic peptide mapping (see Annual Report 1968). Specifically, NH_2-terminal sequences of major urinary protein components 1 and 2, a variant under the control of a single genetic locus (viz., Mup-a) on linkage group VIII, appeared to be identical, whereas component 3 (the most anodal during electrophoresis), exhibited a different NH_2-terminal sequence with a high degree of homology. (Finlayson)

Significance to Bio-Medical Research and the Program of the Division: The control program of the Laboratory requires information regarding the characteristics of proteins and methods used in studying them. The basic research program described above continues to provide the staff with a constantly updated pool of fundamental information concerning the methodology and properties of proteins.

Proposed Course of Project: This is a continuing project.

Honors and Awards: None

Publications: None

Serial No. (NIH-DBS) 176
1. Blood and Blood Products
2. Immunology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Immunosuppression: Anti-Lymphocytic Serum and Rh_o (D) Immune Globulin

Previous Serial Number: Same

Principal Investigator: George W. Morgan, Jr., Ph.D.

Other Investigators: Pinya Cohen, Ph.D.
Elizabeth B. Paul, A.B.
Mildred L. Woodward, B.S.

Man Years:
Total: 1.3
Professional: 1.3
Other: 0

Patient Days: None

Project Description:

Objectives: The overall objectives are to develop potency assays that reflect the efficacy of anti-lymphocytic serum and Rh_o (D) Immune Globulin. Initial objectives include the mechanism(s) of action and optium conditions of production.

Methods Employed: Immunological, serological and physicochemical methods were used.

Major Findings: a) Anti-Lymphocytic Sera: 1) Rabbit antisera to a soluble extract of thymic tissue have been obtained. These antisera will be examined for precipitating and cytotoxic antibody to mouse lymphocytes. 2) Studies on the effect of ALS on the serum protein pattern and efficiency of the RES in antigen clearance are to be initiated.

b) Rh_o (D) Immune Globulin. Data have been collected to determine the normal range of variation of the single dilution radioassay in tests on routine release lots. Data will be collected comparing the sensitivity of a titer scheme involving a series of doubling dilutions with that of the single dilution radioassay. The laboratory is participating in a WHO collaborative study to assess the suitability of a proposed international Rh immunoglobulin reference. The accuracy and precision of the quantitative automated, antiglobulin titer and multiple dilution radioassay methods will be assessed.

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Significance to Bio-Medical Research and the Program of the Division: The Laboratory of Blood and Blood Products is responsible for control of the safety, purity and potency of products derived from blood. This project fulfills these needs for antilymphocytic serum and Rh_o (D) Immune Globulin.

Proposed Course of Projects: This is a continuing project.

Honors and Awards: None

Publications: Esposito, V. M., Paul, E. B., and Seligmann, E. B., Jr.: In Vitro Potency Results from a Collaborative Study of Rh_o (D) Immune Globulin Reference Preparation. Vox Sang. 21:193, 1971.

Esposito, V. M. and Paul, E. B.: Effects of Albumin on the Radio-immune Assay for Determining the Anti-Rh_o (D) Content of Rh_o (D) Immune Globulin. Transfusion 11:281, 1971.

Serial No. (NIH-DBS) 177
1. Blood and Blood Products
2. Immunology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Tissue Typing of the Human Histocompatibility (HL-A) Antigens

Previous Serial Number: Same

Principal Investigator: George W. Morgan, Jr., Ph.D.

Other Investigator: Mildred L. Woodward, B.S.

Man Years:
Total: 1.0
Professional: 1.0
Other: 0

Patient Days: None

Project Description:

Objectives: The overall objective is to develop standards for tissue typing of the human histocompatability antigens. Initial objectives are: 1) development of testing methods, 2) establishment of a reference serum panel, and 3) establishment of a reference cell panel of known specificity.

Methods Employed: Serological, immunological, and physicochemical techniques were used.

Major Findings: a) Additional HL-A antisera have been evaluated and added to the serum panel. This has resulted in an increase in the number of antigens which can be identified, permitting more accurate phenotyping of the cell panel. Additional antisera will be added as the need arises.

b) Improvements in the lymphocytotoxicity assay have been made.

c) The proposed Additional Standards for Leukocyte Typing Serum have been revised and updated.

d) A leukocyte cell panel of known specificity is being established. For those antigens with relatively high phenotypic frequencies, a sufficient number of cells have been identified for routine screening of HL-A antisera. Additional cells will be characterized for addition to this panel.

e) The plausability of freezing panel cells in order to more efficiently utilize donor cells and shorten the man hours required for the assay will be explored.

Significance to Bio-Medical Research and the Program of the Division: The Laboratory of Blood and Blood Products is responsible for control of the safety, purity, and potency of products derived from blood. This project is a part of that responsibility in that the objective is the formulation of standards for tissue typing sera.

Proposed Course of Project: This is a continuing project.

Honors and Awards: None

Publications: None

Serial No. (NIH-DBS) 303
1. Blood and Blood Products
2. Diagnostic Reagents
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Characterization, Stability, and Potency of Diagnostic Reagents

Previous Serial Number: None

Principal Investigators: Clarence F. Szwed, M.S.
Donna C. Williams, B.S.

Other Investigators: Mary C. Strott
Ella F. Miyashiro
Ellis D. Moody, B.S.

Man Years:
Total: 0.7
Professional: 0.5
Other: 0.2

Patient Days: None

Project Description:

Objectives: To obtain detailed information on the characteristics of diagnostic reagents, to evaluate changes in properties which occur during processing and storage, and to develop new methodology and standards.

Methods Employed: Standard serological and immunological methods have been used. Additional specialized methods are described in the following section.

Major Findings: a) A study was undertaken to determine a reliable method of freezing red blood cells in order to have readily available erythrocytes for use in routine control testing. Such erythrocytes must remain antigenically stable throughout the storage period. Using cryogenic techniques, units of freshly drawn blood were frozen and stored in liquid nitrogen. Before freezing the cells were characterized by standardized serological methods. At monthly intervals, aliquots were thawed and subsequently stored at 4 °C. The stored suspensions were tested weekly for four weeks by serological methods for potency and specificity. After three months of frozen storage no differences have been demonstrated in test results with the frozen cells and those obtained prior to freezing. Should this method prove successful, it would eliminate the time consuming and costly need of weekly donor bleedings and the need for ordering erythrocytes from commercial sources when donors with cells of specific antigenic characteristics are required, but are unavailable. (Strott)

b) A method for determining the Anti-Rh_0 (D) content of Rh_0 (D) Immune Globulin is being evaluated. Following a method for quantitating prenatal antibody, the Rh_0 (D) antibody in the current NIH Reference is being studied to determine if reliable and reproducible estimates of antibody content can be obtained. Using a Technicon AutoAnalyzer module for quantitating antibody, the current reference and selected samples are tested in parallel. The results are being compared with those obtained with a radio-immunoassay method. The AutoAnalyzer method if found to be reliable may provide a simplified technique for estimating antibody and in addition would permit more rapid analysis of many samples. (Moody)

c) The nature of and the reasons for particulate matter which appears in diagnostic products is being investigated. These gross particles, only occasionally seen in the past, recently occurred with greater frequency and their appearance was dramatic. Using a variety of chemical methods and physical treatments, the nature of these particles, reasons for their appearance and methods to prevent their formation are being investigated. In addition, the various manufacturing techniques introduced to eliminate or prevent their formation are being evaluated. (Miyashiro)

d) The Additional Standards for Anti-Human Serums are being reviewed. This product is currently marketed in some cases, as being "broad spectrum," i.e., containing both "gamma and non-gamma" components. The latter includes anti-complement activity. Methods for estimating the activity and need for this component and the absolute values to be assigned, if required, are being evaluated. (Szwed)

e) The stability of diagnostic products having undergone significant manufacturing changes is under surveillance. Those products which have been manufactured with a variety of new chemical reagents, and have undergone specific physical treatment, or are in contact with new closures or containers are being studied in terms of stability. This evaluation involves serological determinations of potency and specificity after prolonged storage at constant and ambient temperatures. (Szwed, Moody, Strott and Miyashiro)

f) A preliminary evaluation of the Human Chorionic Gonadotropin unit value as determined by manufacturers and other investigators has been completed. An approximate unit value has been established. A standard unit value will be assigned following the evaluation of various test methods and diluents, and the selection of a standard test procedure chosen for maximum reliability. (Williams)

g) An extensive panel of Human Chorionic Gonadotropin positive and negative urines with clinical follow-up of donors has been established to evaluate the specificity of products submitted for release consideration. The HCG content of selected specimens will be determined and compared with two dried preparations and the second International Standard. (Williams)

h) Immunological methods will be used to evaluate the sensitivity and specificity of Anti-Human Chorionic Gonadotropin products throughout

the dating period. Immunological, chemical and/or physical methods will be used to separate the cells or latex particles from their suspending medium in order to test for unbound HCG or antibody which might affect the stability or specificity of the test after prolonged storage. (Williams)

(i) Immunological and physicochemical methods will be employed to evaluate the effects of medications or their metabolites on the reliability of the products in use for measuring Human Chorionic Gonadotropin. (Williams)

Significance to Bio-Medical Research and Program of the Division: The Diagnostic Reagents Section is responsible for the control of the safety, purity, and potency of diagnostic products derived from a biological source. This surveillance requires a knowledge of product stability, the availability of the most reliable control test methods and technical standards. These projects assist in providing information concerning product stability, dating periods, adequate control test procedures, reference preparations and realistic standards which reflect current knowledge in the area of diagnostic products.

Proposed Course of Project: This is a continuing project.

Honors and Awards: None

Publications: None

Serial No. (NIH-DBS) 304
1. Blood and Blood Products
2. Blood Bank Products
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Characterization, Stability, and Potency of Blood Bank Products

Previous Serial Number: None

Principal Investigators: Madge Crouch
Jeanne Lacerte
Meade Doyle

Other Investigators: Virginia Kane
Ellen Wiles

Man Years:
Total: 0.4
Professional: 0.2
Other: 0.2

Patient Days: None

Project Description:

Objectives: To obtain information on the characteristics of blood products produced in blood banks, to evaluate changes in properties which occur during processing and storage and to develop new or improved methods for measurement of potency.

Methods Employed: Standard serological, immunological and physicochemical methods have been used.

Major Findings: a) A project was initiated to determine if changes occurred in the plasma hemoglobin levels of units of blood stored for certain periods of time at temperatures above 10 °C. The rate of temperature rise in these units and the choice of a reliable plasma hemoglobin test were determined. No conclusive results have been obtained so far with the number of units tested. (Lacerte)

b) During the past year Factor VIII levels were determined in units of Cryoprecipitated Antihemophilic Factor (Human) and Single Donor Plasma (Human) by the performance of Thromboplastin Generation Tests. With the accumulation of data over a period of time statistical analyses are done for the purpose of trying to establish the following:

(1) Average level of Factor VIII found in units of cryoprecipitates.

(2) Comparison of Factor VIII in CPD cryoprecipitates and in ACD cryoprecipitates to determine if CPD cryoprecipitates contain higher level.

Studies were done on the levels of Factor VIII in cryoprecipitates that have been prepared from Single Donor Plasma (Human) stored for 6 months or more. The purpose of this study is to determine if on an average the prolonged storage of plasma before preparing cryoprecipitates has any deleterious effects on the yields of Factor VIII. (Doyle)

Significance to Bio-Medical Research and Program of the Division: This project represents an effort to evaluate certain elements of the processing procedures for selected blood bank products in an effort to improve their quality and potency. It is a rare opportunity to appraise random samples from a variety of sources.

Proposed Course of Project: This is a continuing project.

Honors and Awards: None

Publications: None

CONTRACT NARRATIVE
DIVISION OF BIOLOGICS STANDARDS
July 1, 1971 through June 30, 1972

UNIVERSITY OF OREGON MEDICAL SCHOOL (PH43-67-675)

Title: Investigations on the Mechanisms of Chemical Carcinogenesis

Contractor's Project Director: Dr. A. Wesley Horton

Project Officer (DBS): Dr. Pinya Cohen, LBBP

Assistant Project Officer (DBS): Dr. Carolyn Hardegree, LBP

Objectives: To develop short term biophysical and biological tests to identify carcinogens, promoters of carcinogenesis and to apply such tests to the components of oil adjuvants.

Methods Employed:

(1) C3H and Swiss male mice have been skin-painted and injected subcutaneously with oil adjuvants and their fractions to determine oncogenic activity.

(2) Measurements were made on the enhancement or inhibition of carcinogenesis by promoters and oil fractions on the transport of anionic dye stuffs (simulating metabolites of carcinogens) from an aqueous to an organic phase, mediated by phospholipids and cholesterol.

(3) Measurements were made of the surface tension of a reference monomolecular film of cholesterol compared to values obtained when carcinogens, adjuvant components and noncarcinogens were mixed with cholesterol. Test components showing unusual effects in this system were subjected to skin-painting experiments in mice to determine if the biophysical data could be confirmed.

Major Findings: Skin-painting experiments employing an initiation-promotion protocol have shown that the most active inhibitors of tumor formation are the medium refractive index materials of Component No. 2 (a blend oil for white mineral oil) and the major component of peanut oil - triolein. Component 1 was found to be the most active accelerator of carcinogenesis.

Component No. 1, a low viscosity oil used to prepare white mineral oil (Drakeol 6VR), is primarily responsible for the induction of sarcomas in Swiss, male mice, however, other oil fractions induce sarcomas. The average latent period is being determined for each of these fractions.

Mineral oil accelerates the rate of skin carcinogenesis produced by a chemical carcinogen.

Arlacel A emulsifier given subcutaneously promotes the induction of sarcomas in Swiss, male mice.

Biophysical systems simulating cell membranes showed that paraffins producing maximum inhibition of interfacial transport of a dye simulating metabolites of carcinogens correlated with the greatest carcinogenic response in mice.

Monomolecular films react selectively with moderate and strong carcinogens but not with incomplete or noncarcinogens.

Significance to DBS Program and Biomedical Research: These studies are designed to develop quick biophysical tests to determine the presence of carcinogens in components of oil adjuvants; elucidate mechanisms of action of carcinogenesis and establish criteria for the safety of adjuvant preparations.

Proposed Course of Project: The contract will terminate. September 30, 1972.

Date Contract Initiated: March 17, 1967

Current Annual Level: $34,540

Publication: Horton, A.W. and McClure, D. W.: Ionic transport through model membranes I. Effects of accelerators of carcinogenesis. Biochimica Biophysica Acta. 225:248-253, 1971.

CONTRACT NARRATIVE
DIVISION OF BIOLOGICS STANDARDS
July 1, 1971 through June 30, 1972

IIT RESEARCH INSTITUTE (PH43-67-1101)

Title: Chemical and Physical Analysis of Oils and Emulsifiers

Contractor's Project Director: Dr. Hugh J. O'Neill

Project Officer (DBS): Dr. Pinya Cohen, LBBP

Assistant Project Officer: Dr. Carolyn Hardegree, LBP

Contract Expired: June 22, 1969

Publications: O'Neill, H. J., Yamauchi, T. N., Cohen, P. and Hardegree, M. C.: Characterization of mannide monooleate. J. Pharmaceutical Sciences. In press.

O'Neill, H. J., Yamauchi, T. N., Cohen, P. and Hardegree, M. C.: Characterization of adjuvant mineral oils. J. Pharmaceutical Sciences. In press.

Annual Report of the
Laboratory of Control Activities
Division of Biologics Standards
July 1, 1971 through June 30, 1972

I. Mission

The Laboratory of Control Activities has prime responsibility within the Division for performing tests on biological products to determine their conformance to standards of safety, purity and potency. The Laboratory is composed of three sections: Analytical Chemistry, Control Test and Reference Standards.

The Laboratory has the following functions:

(a) To conduct chemical, sterility, general safety, pyrogen, residual moisture and potency tests on biological products submitted for release or in support of license applications. Of all the various licensed products, the approximate number tested by the Laboratory is 300 for sterility, 200 for general safety, 100 for potency, 100 for chemicals, 50 for pyrogen and 40 for residual moisture. This was accomplished by performing 5243 tests for sterility, 941 tests for general safety, 893 tests for potency, 794 tests for chemicals, 537 tests for pyrogens and 536 tests for residual moisture. Testing is accompanied by review of the manufacturers' protocols with respect to the tests performed by the Laboratory. Applied research is conducted on development and modification of control tests on biological products.

(b) To develop, maintain and distribute biological standards, references, control materials and reagents necessary for the testing of products to determine compliance with published standards. A culture collection is also maintained to support the technical effort of the Division as well as to support control activities as they affect the licensed manufacturers.

(c) In addition to the above, the Laboratory shares responsibility with the other Laboratories in the Division in developing standards and regulations, performing inspections, reviewing license applications and maintaining a technical liaison with the World Health Organization, other national control agencies and recognized authorities in the field of biological products.

II. Product Testing

Individual lots of licensed products are controlled by the Division through a system of evaluation and release utilizing data submitted by the manufacturer and that developed by the testing program of the Laboratory of Control Activities and other testing areas of the Division. All testing is directed toward the objective of assuring that products meet prescribed standards of safety, purity and potency.

Included in the purity testing of biological products are tests for pyrogenicity, residual moisture and specific chemicals, which also serve as means for evaluating and controlling the safety and stability of such products.

The types of products tested by the Laboratory include antitoxins and therapeutic immune serums, blood and blood derivatives, bacterial vaccines, toxins and toxoids, multiple antigens, viral and rickettsial vaccines, diagnostic substances for dermal tests, antivenins, venoms, bacterial enzymes and allergenic extracts.

In addition to the control testing of biological products within the Division, the Control Test Section of the Laboratory of Control Activities provides regular cooperative pyrogen testing services to the Clinical Center Pharmacy, Blood Bank and Media Department and sterility testing for the Clinical Center Pharmacy and Blood Bank.

A summary of 14,950 tests performed by the Laboratory during a 12 month period (April 1, 1971 through March 31, 1972) in making the determination that products meet prescribed standards is shown in Table 1.

TABLE I

Samples Tested	Number of Tests Performed				
	Sterility	Safety	Potency	Purity	Total
Products for Release	5,243	941	893	1,867	8,944
Inspection	268	0	4	34	306
Cooperative Service	2,216	0	6	354	2,576
Reference and Standardization	19	1	325	14	359
Complaint	39	27	35	13	114
Research and Development	175	6	1,092	1,378	2,651
Totals	7,960	975	2,355	3,660	14,950

Note: Of the 7,960 total sterility tests, 1,150 (14.4%) were performed for the Clinical Center Pharmacy and 1,020 (12.8%) were performed for the Clinical Center Blood Bank. Also, 35% of all pyrogen tests were performed for the Clinical Center Pharmacy.

III. Standard Preparations

An integral part of the system of manufacture and control of biological products is the application and use of standard preparations. By the use of such standards, potency of a product can be determined and standardized so as to give increased assurance of consistency from lot to lot. It is the function of the standards program of the Laboratory to provide these standard and reference preparations so that the control program of the Division as well as manufacturers and others engaged in testing may have available the tools to calibrate and evaluate products. It is largely through use of these preparations that those portions of the biologics control regulations relating to potency can be applied.

In order to maintain an adequate supply of standards and references it was necessary to prepare and calibrate during the past year the following lots of materials: 12 antitoxins, 1 serum, 1 vaccine, 6 miscellaneous preparations. In addition to these materials prepared by the Laboratory the following lots of materials were purchased from commercial sources and finished by the Laboratory: 8 virus vaccines, 9 bacterial vaccines, 8 allergenic products, 1 blood typing serum, and 121 hepatitis associated antigen containing sera. A total of 359 tests were required to complete a satisfactory standardization of these materials and maintain surveillance of the stability of all preparations. These tests included such procedures as flocculation reactions, animal protection tests, animal titrations, neutralizations and assorted in vivo and in vitro tests.

The technique for freeze-drying is a key procedure applied in the preparation of standard and reference materials whenever possible in order to increase assurance of stability over long periods of storage. The following numbers of ampules of various preparations were dried during the year.

Cultures	3,209	ampules
Serums	1,267	"
Viruses	40	"
Plasma	4,467	"
Total	8,983	"

The following standard and reference preparations, consisting of a total of 1,115 separate shipments were distributed to manufacturers, P.H.S. and state health laboratories, universities, foreign manufacturers holding U. S. licenses and qualified foreign laboratories.

Australia Antigen	14,021
Antitoxins	486
Serums	4,950
Vaccines	2,498
Toxins	531
Bacterial & Viral Cultures	282
Allergens	425
Viruses	1,713
Antigen E	53

Opacity Standard	90
Globulins	329
Blood Products	920
Miscellaneous	1,348
Total	27,646

Approximately 120 different standards, references, and related control preparations are maintained for distribution.

IV. Related Activities

In addition to the effort outlined above, the laboratory staff also participated during the year in a number of ancillary activities directly related to control which accounted for a substantial segment of its working time.

(a) Members of the professional staff of the Laboratory were members of 66 committees for the review of product and establishment license applications submitted to the Division. In addition 30 reviews of submissions to Investigational New Drug (IND) applications were made.

(b) The Laboratory staff participated in 60 of the on-site inspections required to be performed annually on all licensed establishments. Approximately 69 man days of work were consumed during the year in this effort.

V. Collaboration with World Health Organization

The Laboratory of Control Activities works closely with the biological standardization program of the World Health Organization. Working with Dr. Margaret Pittman (retired) a collaborative study for WHO was performed on opacity preparations. Serving as a reference testing laboratory for cholera vaccine for WHO, four lots of vaccine have been tested. These are samples of vaccine produced in various foreign countries. The World Health Organization uses this method of surveillance to evaluate the quality of vaccines produced in countries having no internal national control program. The quality of vaccines produced in such countries has a direct bearing on the safety of U.S. citizens working and traveling abroad.

VI. Other Activities and Honors

Edward B. Seligmann Jr., Ph.D.	Panel Member of the World Health Organization Expert Committee on Biological Standardization
	Temporary Advisor for Formulation of International Requirements for Rabies Vaccine, World Health Organization
	Member FDA Task Force on Use of Antibiotics in Animal Feeds
	Member of U.S. Pharmacopeia Advisory Panel on Microbiological Attributes and Procedures

	NIAID, Collaborative Research, Research Resources Branch, Consultant for Freeze-drying and Reagent Packaging Contracts
	Member of PHS Postal Service Advisory Committee
	PHS Observer for Destruction of Biological Warfare Agents
Lee J. Stevan, M.S.	Member of the Executive Committee of the Interagency Botulism Research Coordinating Committee.

VII. Research and Development

While the primary function of the Laboratory of Control Activities is concerned with various aspects of controlling the safety, purity and potency of biological products, a substantial research program is conducted in order to provide a sound basis for the testing program and selection of preparations for use as official standards and references. The following are summaries of some of the research conducted by Laboratory personnel during this fiscal year.

(a) Opacity Standard: The U. S. Opacity Standard which is a suspension of fine glass particles in water, is used to adjust bacterial vaccines to required concentrations and to adjust the cell content of challenge suspensions used in potency tests. Currently, these comparisons are determined in different types of instruments that are designed to measure density. Because of its composition there are inherent problems concerning stability and reproducibility of lots having consistent properties. During storage and use, the density of the preparation is gradually reduced by loss of glass particles from the suspension due to adherence to the sides of the container. To overcome these problems, experimental lots have been prepared by the suspension of glass particles in a semisolid resin. Although such a preparation would provide a greater degree of stability, it presents problems of a different nature. Because heat must be applied, to a limited degree, to liquefy the resin, the mechanics of particle suspension, mixing and dispensing add to the difficulty of preparing a reasonably large lot having a neglible variation in optical density from tube to tube. An additional problem to be resolved, concerns the method of transferring the resin preparations from the container in which it is distributed into suitable colorimeter tubes designed specifically for use in each of a variety of instruments.

The possibility of using the current type of preparation in determining density by visual means i.e., without the use of an instrument, has also been evaluated. Difficulties have been encountered in this method due to the presence of color that tends to influence density comparisons made by eye. Color does not interfere with determinations by instrument.

The U. S. Opacity Standard, as it is presently distributed, has continued to be used successfully for a number of years in the production of bacterial vaccines and preparation of challenge suspensions. Until the problems encountered in preparing a different type of standard are resolved, the

U. S. Opacity Standard will continue to be prepared by the same proven method and distributed for use in comparative density determinations by instrument.

(b) Sterility Testing: Several aspects of sterility testing are being studied with the object of improving the validity of sterility tests and reducing the time, volume of medium and cost involved in performing each test. New and modified media are being studied using selected microorganisms having peculiar growth characteristics. Methods for using membrane filtration for testing a variety of products are being developed. An improved cutter for dividing the membrane in two exactly equal portions was developed. An instrument capable of detecting contamination automatically in biological samples was investigated. Although the numerous mechanical and electronic problems which developed were quickly and efficiently resolved, the unexpected biological problems which developed could not be resolved without redesigning the instrument and/or developing an acceptable radioactive medium. Since neither of these areas of study is within the purview of this Laboratory, the instrument was returned to the manufacturer. In addition, the instrument was not capable of detecting anaerobic organisms or non-glucose fermenting organisms within a period of time shorter than that required by routine laboratory techniques.

(c) Coccidioidin: Under two contracts and with LCA and LBP, a collaborative study is in progress to develop a satisfactory reference preparation for coccidioidin and a suitable laboratory potency test. This work will be correlated with responses in man.

(d) Typhus Vaccine: Vaccines prepared in a series of four serial two-fold dilution levels of activity have been obtained under contract with a licensed manufacturer. The four preparations have been tested in laboratory studies for safety, potency and purity. They possess an extremely high level of purity. A clinical study with these vaccines has been initiated at Camp Lejeune, North Carolina and sera from the first phase of the study are being titered. The project on Typhus Vaccine is being conducted in collaboration with the Laboratory of Virology and Rickettsiology.

(e) In Vitro Test for Diphtheria Antitoxin: Studies are in progress on the use of cell cultures for titrating diphtheria antibody. The results look promising and the procedure offers the possibility of improved sensitivity over that of the present animal test.

(f) Pyrogen Testing: Studies are in progress evaluating the Limulus test for endotoxin as a possible rapid in vitro test for pyrogens in biological products. The test is extremely sensitive for endotoxins, is rapid, and is inexpensive. It is very promising as a substitute for the expensive and time consuming in vivo pyrogen test. However, there are a number of critical details of the test procedure which must be studied and standardized before it will be suitable for routine use. So far no material has been found that is pyrogenic to rabbits and not detected by the Limulus test.

Serial No. DBS 29
1. Control Activities
2. Analytical Chemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Development of Methods for the Chemical Analysis of Biological Products.

Previous Serial Number: Same

Principal Investigator: Edward B. Seligmann Jr., Ph.D.

Other Investigators: J. Richard Miller
Roscoe M. Wheeler

Cooperating Units: None

Man Years

Total: 2.25
Professional: 1.25
Other: 1.0

Project Description:

Objectives: To develop new methods of chemical analysis of biological products or to modify existing methods, thus facilitating the study of chemical problems in the control of and research on biologicals.

Methods Employed: Standard methods of chemical analysis are used, evaluated, or modified as required for the material being analyzed, and new methods are developed.

Major Findings: An extensive program is now under way to analyze dried biologicals by the traditional gravimetric method. This program will be expanded to investigate the possibility of using a micro gravimetric method or other available methods such as gas chromatography, Karl Fischer, and infrared spectroscopy for determining of moisture in dried biologicals.

A study is in progress to test for residual moisture in all dried biological products at the beginning and periodically throughout the dating period. Several storage temperatures are being studied for selected lots.

Significance to Bio-medical Research and the Program of the Division: The significance of this work lies in providing increased knowledge of the chemical composition of biologicals which can be of value in research, and in

surveillance of these products for the protection of the public.

Proposed Course of Project: The project will be continued with the addition of the following programs:

1) Expansion of the study of the amount of mercury assayed in all biologicals which have the mercurial preservative thimerosal (Merthiolate, Lilly) by a method developed in the Analytical Chemistry Section. These biological samples will be assayed through the entire dating period to determine if there is any change in the thimerosal level during storage. In addition, work will be initiated on developing methods or modifying existing methods for the thimerosal assay.

2) A Reference L-Asparaginase preparation is being procured and will be standardized for use in the assay of enzyme activity and disc electropheretic patterns.

3) Work will be continued on the development of a method to measure alum and aluminum phosphate in biologicals by atomic absorption spectrophotometry.

Honors and Awards: None

Publications: None

Serial No. DBS 103
1. Control Activities
2. Reference Standards
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Standardization of Coccidioidin

Previous Serial Number: Same

Principal Investigators: Vernon J. Fuller, Ph.D.
Edward B. Seligmann Jr., Ph.D.
Sotiros D. Chaparas, Ph.D.

Other Investigator: Ruben E. Marquina

Cooperating Units: Laboratory of Bacterial Products

Man Years

Total: 0.50
Professional: 0.25
Other: 0.25

Project Description:

Objectives: To develop suitable laboratory procedures and reference preparations for use in the potency testing of coccidioidin.

Antigens from spherule and mycelial growth of several strains of Coccidioides immitis prepared on contracts have been analyzed by acrylamide electrophoresis and homologous and heterologous skin tests.

Major Findings: Three of six strains have been investigated thus far and acrylamide patterns and cross skin reactions suggest a great deal of antigenic similarity.

Significance to Bio-medical Research and the Program of the Division: (1) The apparent similarities of strains of Coccidioides immitis may mean that the type or number of strains used to prepare coccidioidin for skin testing may not be very important. (2) These results will be helpful in determining and characterizing materials to be used as a suitable standard preparation for Coccidioidin. This will permit the Division to better control commercial preparations for uniformity and consistency of production.

Proposed Course of Project: (1) Three more strains will be studied and evaluated with the three previous strains. (2) With information gathered in DBS Laboratories and on contract a new standard for Coccidioidin will be

prepared and evaluated.

Honors and Awards: None

Publications: Chaparas, S. D., Fuller, V. J. and Seligmann, E. B. Jr.: Laboratory Procedures for Potency Testing of Blastomycin, Histoplasmin and Coccidioidin. Proc. Soc. Exp. Biol. Med. (Accepted for publication)

Serial No. DBS 144
1. Control Activities
2. Reference Standards
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Clostridium botulinum Toxin and Antitoxin

Previous Serial Number: Same

Principal Investigator: Lee J. Stevan, M.S.

Other Investigator: Edward B. Seligmann Jr., Ph.D.

Cooperating Units: Laboratory of Pathology

Man Years

Total: 1.5
Professional: 1.0
Other: 0.5

Project Description:

Objectives: To establish the relationship of various nutritional components of the growth medium to the biochemical structure of toxins of various strains of Clostridium botulinum types A, B, C, E, and F.

Methods Employed: Techniques of microbiology and electron microscopy.

Major Findings: Previous studies showed that growth and toxin production of C. botulinum was affected by the addition of glucose on EDTA to the growth medium. The variation of cellular morpology as studied under scanning electron microscopy were shown to reflect the conditions under which they were grown.

Significance to Bio-medical Research and the Program of the Division: This program should help explain the reasons why some lots of test toxins are inadequate. The potency testing of botulism antitoxins could be supplemented by the results of this project.

Proposed Course of Action: The results of this study will be evaluated and the project will be terminated at the end of the year.

Honors and Awards: None

Publications: Ewell, John B., McGrath, Philip P., Stevan, Lee J., Wehrung, John M.: Scanning Electron Microscopy of Clostridium Botulinum Under Varying Growth Conditions. Electron Microscope Society of America, 1972. In press.

Serial No. DBS 145
1. Control Activities
2. Reference Standards
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Coral Snake Venom and Antivenin

Previous Serial Number: Same

Principle Investigator: Edward B. Seligmann Jr., Ph.D.

Other Investigators: Lee J. Stevan, M.S.
William H. Berkeley

Cooperating Units: None

Man Years

Total: 1.0
Professional: 0.5
Other: 0.5

Project Description:

Objectives: Establishment of standards for coral snake antivenin. Study of the immunogenic properties of coral snake venoms from various species of snakes. Electrophoretic studies of coral snake venoms.

Methods Employed: Hyperimmunization of various species of animals. Venom neutralization tests. Immunoelectrophoresis, agar gel diffusion, acrylamide disc electrophoresis and agar gel electrophoresis.

Major Findings: Studies of the degree of cross neutralization of venom from various species of coral snakes by several commercial coral snake antivenins and some experimental specific antisera are in progress. Studies on venom from the Arizona coral snake (Micruroides euryxanthus) are being conducted with the small amount of available venom being the limiting factor.

Significance to Bio-medical Research and Program of the Division: As an outcome of work performed in the Laboratory, Wyeth Laboratories was licensed in September 1967 for Antivenin (Micrurus fulvius) which is effective against the venom of all coral snakes found in the United States except for Micruroides euryxanthus, the Arizona coral snake. Studies on cross-neutralization of venom from various species of coral snake are leading to an understanding of the extent of usefulness of coral snake antivenins for treatment of bites from coral snakes found throughout North, Central and South America.

Proposed Course of Project: Completion of work in progress on cross-neutralization and immunochemical properties of venoms from various species. Completion of work in progress on the in vivo effectiveness of antivenins in treating envenomation. Study of venom from the Arizona coral snake.

Honors and Awards: None

Publications: Cohen, P., Berkeley, W.H., and Seligmann, E.B. Jr.: Coral Snake Venoms: In Vitro Relationship of Neutralizing and Precipitating Antibodies. The Amer. J. of Trop. Med. and Hyg. Vol. 20, No. 4: p. 646-649, July 1971.

Serial No. DBS 147
1. Control Activities
2. Reference Standards
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Development of an Improved Opacity Standard

Previous Serial Number: Same

Principal Investigator: Edward B. Seligmann Jr., Ph.D.

Other Investigator: Jane F. Farber

Cooperating Units: None

Man Years:

Total: 0.15
Professional: 0.15
Other: 0

Project Description:

Objectives: 1) To prepare a stable solid opacity standard by suspending finely divided particles in a resin.

2) To standardize this preparation in terms of the current opacity standard which is an aqueous suspension of glass particles.

3) To determine the practicality of distributing a solid standard for use by manufacturers.

Methods Employed: Varying concentrations of titanium dioxide or glass particles are evenly suspended in viscous melted resin and allowed to harden in selected tubes. The concentration having the same light transmission as the U.S. Opacity Standard is then selected.

Major Findings: The problems encountered in preparing large numbers of tubes of the resin preparation with identical optical density make it unlikely that this type of preparation will be an improvement over the present Opacity Standard.

Significance to Bio-medical Research and the Program of the Division: The U.S. Opacity Standard is used to standardize the cell concentrations of bacterial vaccines and to achieve lot to lot consistency. The bacterial cell concentration of challenge doses used in potency tests are also adjusted

with this standard.

Proposed Course of Project: The U.S. Opacity Standard as it is presently distributed has continued to be used successfully for a number of years, in the production of bacterial vaccines and preparation of challenge suspensions. Until the problems encountered in preparing a different type of standard are resolved, the U.S. Opacity Standard will continue to be prepared by the same proven method and distributed for use in comparative density determinations by instrument.

Honors and Awards: None

Publications: None

Serial No. DBS 150
1. Control Activities
2. Reference Standards
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Replacement of the U.S. Standard Tetanus Antitoxin

Previous Serial Number: Same

Principal Investigator: Jane F. Farber

Other Investigator: Edward B. Seligmann Jr., Ph.D.

Cooperating Units: None

Man Years

Total: 0
Professional: 0
Other: 0

Project Description:

Objectives: To select a new lot of antitoxin that will prove suitable for use as a master dried U.S. Standard Tetanus Antitoxin, the supply of which is nearly depleted.

Methods Employed: The protective unitage relative to the master U.S. Standard Tetanus Antitoxin was determined by comparative neutralization tests in mice and guinea pigs.

Antitoxin characteristics such as avidity and heat stability were defined by the following methods using two toxins:

1) Comparison in mice of the L+ values obtained at test levels of L+/10, L+/100 and L+/1000.

2) Stability of toxin-antitoxin mixtures following periods of incubation at room temperature.

3) Comparison of the L+ values of glycerinated preparations stored at 37°C.

Major Findings: A dried preparation of tetanus antitoxin has been selected as the new master U.S. Standard Tetanus Antitoxin to replace the first master preparation which is now depleted. Through in vivo tests in this laboratory of several glycerinated lots in addition to the findings submitted

by the licensed manufacturers following their evaluation of a glycerinated lot, the suitability of this preparation as the new master antitoxin has been established.

Proposed Course of Project: Project is completed.

Honors and Awards: None

Publications: Seligmann, E.B. Jr. and Farber, J.F. Freeze Drying and Residual Moisture: Cryobiol. 8:138-144, 1971.

Serial No. DBS 153
1. Control Activities
2. Reference Standards
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Development of a Reference Typhus Vaccine and an Improved Potency Test

Previous Serial Number: Same

Principal Investigator: Edward B. Seligmann Jr., Ph.D.

Other Investigators: Otis L. Green
Harry T. Aylor
Elmer C. Russell

Cooperating Units: Laboratory of Virology and Rickettsiology

Man Years

Total: 0.25
Professional: 0.15
Other: 0.1

Project Description:

Objectives: Establishment of a suitable Reference Typhus Vaccine preparation. Development of an improved potency test for typhus vaccine.

Methods Employed: Standard potency test described in the Minimum Requirements for Typhus Vaccine and a variety of modifications including antigen extinction type tests.

Major Findings: No modifications of the animal potency test have been found that are significantly better than the official test. However the test is not sensitive enough to differentiate between vaccines known to have a two-fold difference in antigen content. The complement fixation test is somewhat more sensitive, but not entirely satisfactory because of several very critical variables in the test. A clinical study is being performed at Camp Lejeune, North Carolina by Richard P. Wenzel, M.D. using four vaccines prepared as two-fold serial dilutions of a parent lot. Sera from recipients of the two most dilute vaccines are being titered prior to completing the study with the two higher concentrations.

Significance to Bio-medical Research and the Program of the Division: The effectiveness of present typhus vaccine has been questioned by the

scientific community concerned with the use of typhus vaccine in the armed services. A reference vaccine would aid in assuring more consistency among production lots. In addition, the correlation between the animal test and effectiveness in man must be established.

Proposed Course of Project: Under contract, typhus vaccine at four two-fold gradations in titer has been obtained and is being used in correlating laboratory assay procedures and clinical response.

Honors and Awards: None

Publications: None

Serial No. DBS 168
1. Control Activities
2. Reference Standards
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Malayan Pit Viper Venom

Previous Serial Number: Same

Principal Investigators: Lee J. Stevan, M.S.
D. L. Aronson, M.D. (LBBP)

Other Investigator: Edward B. Seligmann Jr., Ph.D.

Cooperating Units: Laboratory of Blood and Blood Products

Man Years

Total: 0
Professional: 0
Other: 0

Project Description:

Objectives: To describe and evaluate the anti-coagulant and thrombolytic activity of Malayan pit viper (Agkistrodon rhodostoma) venom.

Methods Employed: Chromatography and electrophoresis, necessary tests used to evaluate the various venom fractions such as fibrinolysis, hemorrhagic activity and in vitro clotting of fibrinogen.

Major Findings: No work was undertaken because of delays in the time schedule of the manufacturer developing the product. Preliminary work by DBS has been completed and further work is dependent upon the characteristics of the product to be manufactured.

Significance to Bio-medical Research and the Program of the Division: This investigation will lead to an understanding of the anti-coagulant and thrombolytic effects of Malayan pit viper venom. This understanding will in turn help the Division evaluate the potency and purity of any new drugs derived from this venom.

Proposed Course of Action: Development of suitable reference preparations and necessary supportive work after the manufacturer submits a product.

Honors and Awards: None

Publications: None

Serial No. DBS 169
1. Control Activities
2. Reference Standards
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Comparison of Titrations in Monkey Kidney Tissue Cells and Chorioallantoic Membrane of Chick Embryos for the Potency Assay of Smallpox Vaccine.

Previous Serial Number: Same

Principal Investigator: Vernon J. Fuller, Ph.D.

Other Investigator: Ruben E. Marquina

Man Years

Total: 0
Professional: 0
Other: 0

Project Description:

Objectives: To compare the potency of the U.S. Reference as determined by the chorioallantoic membrane method and the monkey kidney tissue cell (MKTC) method.

Methods Employed: The chorioallantoic membrane technique and MKTC method were employed in this study.

Major Findings: This project was held in abeyance during the current year due to priority given other studies.

Significance to Bio-medical Research and the Program of the Division: If the variability of the procedure can be brought under control, a third potency test method applicable to the standardization of smallpox vaccines may result from this study.

Proposed Course of Project: This study will be continued as time permits.

Honors and Awards: None

Publications: None

Serial No. DBS 178
1. Control Activities
2. Control Test
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Electron Microscopy of Vaccinia Virus Produced Under Conditions of Amino Acid Deprivation.

Previous Serial Number: Same

Principal Investigator: Edward A. Fitzgerald, Ph.D.

Other Investigator: Philip P. McGrath

Cooperating Units: Laboratory of Pathology

Man Years

Total: 0
Professional: 0
Other: 0

Project Description:

Objectives: To investigate the morphology and development of vaccinia virions produced by host cells which are nutritionally deprived because of the omission of certain amino acids from the culture medium.

Methods Employed: The majority of virus particles produced under these conditions are not fully mature and only a small percentage are infectious. Electron microscopic examination of thin sections of infected cells which were fed with depleted medium show virus particles with apparent abnormalities in the structure of the outer coat. Further work will involve purification of the virus yield from these depleted cells using a sucrose density gradient. The gradient may then be fractionated and electron microscopy performed on certain of these fractions.

Major Findings: Only preliminary examinations of infected cells have been completed because of the time limitations imposed by work of higher priority.

Significance to Bio-medical Research and the Program of the Division: This study will contribute to a further understanding of vaccinia virus morphogenesis and the effects of nutritional depletion on virus replication.

Proposed Course of Project: This study has been terminated.

Honors and Awards: None

Publications: Ph.D. Thesis: Fitzgerald, E.A., Amino Acid Requirements in Vaccinia Virus Biosynthesis, Catholic University. Abstract published in
(1) Dissertation Abstracts International, Vol. XXXI, No. 5, 1970
(2) Bacteriological Proceedings. 1970, p. 196, No. V278

Serial No. DBS 180
1. Control Activities
2. Reference Standards
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Comparison of Media Used in Sterility Testing

Previous Serial Number: Same

Principal Investigator: Vernon J. Fuller, Ph.D.

Other Investigators: Edward B. Seligmann Jr., Ph.D.
Ruben E. Marquina

Cooperating Units: None

Man Years

Total: 1.25
Professional: 0.75
Other: 0.5

Project Description:

Objectives: To compare the growth-promoting qualities of four media used in sterility testing. Fluid Thioglycollate medium; Fluid Sabouraud medium, pH 5.7; Fluid Sabouraud medium, pH 7.1 and Soybean-Casein Digest medium are being compared.

Methods Employed: Aliquots of serial dilutions of aerobic and anaerobic fungal and bacterial cultures were inoculated into replicate tubes of the four test media. One set of tubes was incubated at 22° C. and the other at 31° C. for 10 days or longer depending upon the test organism.

Major Findings: A) Four of the five fungal cultures studied grew readily at both temperatures; Cephalosporium acremonium, Mucor mucedo, Candida albicans, and Saccharomyes cerevisiae. More luxurious growth was observed in cultures of Aspergillus niger when incubated at 31° C.

The fungi studied grew equally well in each of the four media studied at their respective optimum temperature.

B) In this study it was found that the optimum temperature for the 20 bacterial cultures used was 31° C. and that under these conditions:

1) Fluid Thioglycollate medium is the medium of choice for the propagation of both the aerobic and anaerobic organisms studied.

2) The modified Sabouraud medium and Soybean-Casein Digest medium are of equal value for growth of the organisms.

3) Fluid Sabouraud medium was of less value in propagating the bacteria. However, one must remember that Sabouraud's medium was developed for the detection of mold, yeast, acidophilic bacteria and that the acid reaction of the medium is inhibitive to a large number of bacteria.

Significance to Bio-medical Research and the Program of the Division: The data obtained will aid in evaluating the media used in sterility testing by the Division and those used by other investigators.

Proposed Course of Project: Additional fungal cultures will be studied. The results of this study will be prepared for publication.

Honors and Awards: None

Publications: Russell, E.C., Farber, J.F., Seligmann, E.B. Jr.: Cutter for Membrane Filter Used in Sterility Testing. Appl. Microbiol. 21:1110-1111, 1971.

Serial No. DBS 208
1. Control Activities
2. Reference Standards
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Development of a Reference Tetanus Toxoid

Previous Serial Number: Same

Principal Investigator: Jane F. Farber

Other Investigator: Edward B. Seligmann Jr., Ph.D.

Cooperating Units: Laboratory of Bacterial Products

Man Years:

Total: 0.5
Professional: 0.5
Other: 0

Project Description:

Objectives: To select a reference tetanus toxoid that will prove most useful in determining the relative antigenic activity of commercially prepared toxoids. The following aspects of the problem will be investigated:

1) Relationship of in vitro and in vivo potency values.

2) Effect of varying amounts of mineral adjuvants combined with toxoids of varying potency.

3) Relationship of antigenic activity as determined in animals to the values obtained in human studies.

4) Determination of protective unitage against challenges of different toxins.

Methods Employed: The antigenic activity of fluid and adsorbed toxoids in guinea pigs and mice will be measured by the direct toxin challenge method and by neutralization test of serum antibodies. These findings will be correlated with activity determined in clinical studies. In vitro activity will be determined by the flocculation test.

Major Findings: Results of a limited number of in vivo tests comparing the potency of three toxoid preparations which have equivalent in vitro activity but vary as to the concentration of aluminum phosphate adjuvant are summarized below:

1) Higher concentrations of adjuvant give rise to an increase in the protective level of the toxoid in mice and guinea pigs as measured by the challenge test.

2) The potency values of the toxoids containing adjuvant are significantly higher in guinea pigs than in mice when determined by the challenge test method.

3) The percentage of guinea pigs that produce 2u/ml. of serum antibody in response to fractional doses of toxoid ie. <0.50 ml., increases with the adjuvant content of the toxoids.

Significance to Bio-medical Research and the Program of the Division: The precision of direct quantitative measurement of the potency of commercially prepared toxoids will be improved by comparative testing with a reference toxoid. The development of a laboratory test method which most closely correlates with the potency determined in human studies will enhance the accuracy of comparative testing.

Proposed Course of Project: To investigate all aspects of laboratory *in vivo* and *in vitro* test methods of measuring the immunizing activity of fluid and adsorbed tetanus toxoids and to correlate these findings with those determined in clinical studies.

Honors and Awards: None

Publications: None

Serial No. DBS 305
1. Control Activities
2. Control Test
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Development of an In Vitro Potency Test for Diphtheria Antitoxin

Previous Serial Number: None

Principal Investigator: Edward A. Fitzgerald, Ph.D.

Other Investigator: Walter S. Hunter

Cooperating Units: None

Man Years

Total: 0.3
Professional: 0.15
Other: 0.15

Project Description:

Objectives: To develop a suitable in vitro potency test for diphtheria antitoxin and to correlate this with the official test performed in rabbits.

Methods Employed: The cell culture test used in Holland by the Rijks Instituut voor de Volksgezondheid has been modified for use in this study. A number of different cell types have been used in an attempt to find the one most sensitive to the toxin-antitoxin system.

Major Findings: The choice of a suitable diphtheria toxin and antitoxin has been made after testing several lots of each material. The working levels of each of these products has also been established by titrating varying concentrations of toxin and antitoxin. Both U. S. Standard antitoxin and WHO antitoxin were tested against a number of different toxins.

Of the cell types tested so far, primary rabbit kidney cells appear to be most suitable for this test. The results obtained by this method seem to correspond to those from the rabbit test but further testing is needed to clarify this point.

Significance to Bio-medical Research and the Program of the Division: The study may yield a more sensitive potency test which will be easy to perform and free from the variation often seen in animal testing.

Proposed Course of Project: Further studies will be made on the correlation between this test and the official rabbit test. Also, the study will be expanded to encompass the use of both human and non-human diploid cells as the indicator system.

Honors & Awards: None

Publications: None

Serial No. DBS 306
1. Control Activities
2. Control Test
3. Bethesda, Maryland

PHS-NIH
Individual Project Reports
July 1, 1971 through June 30, 1972

Project Title: Studies on the Improvement of the Rabies Vaccine Potency Test

Previous Serial Number: None

Principal Investigator: Edward A. Fitzgerald, Ph.D.

Other Investigators: Walter S. Hunter
Otis L. Green

Cooperating Units: None

Man Years

Total: 1.2
Professional: 0.7
Other: 0.5

Project Description:

Objectives: To investigate alternate methods for determining the potency of Rabies Virus Vaccine both *in vivo* and *in vitro*.

Methods Employed: The antibody-binding test, developed at the Center for Disease Control, Atlanta, Georgia, has been used extensively in this study. A large number of lots of Rabies vaccine submitted for release have been tested using this method in comparison with the official NIH potency test. Plaque assay studies using BHK-21 cells have also been initiated.

Major Findings: The correlation between the official NIH potency test and the antibody-binding test (*in vivo*) appears to be very good. This test may be better than the present one for testing the potency of cell culture rabies vaccines which are being developed by some manufacturers.

In vitro tests using the antibody-binding technique are being attempted but these are still in the preliminary stage and have yielded no significant data.

Significance to Bio-medical Research and the Program of the Division: The present potency test for Rabies Vaccine may not be adequate for testing the new cell culture vaccines now under development. This study may result in the improvement of this test in terms of both speed and accuracy. Successful development of an *in vitro* test could also eliminate the variability so often

seen in animal testing.

Proposed Course of Project: The in vivo testing will continue to correlate results with the official N.I.H. potency test and appropriate statistical tests will be applied to determine the significance of these results. Cell culture testing will be emphasized in an effort to establish the relationship between this test and the two in vivo tests.

Honors and Awards: None

Publications: None

Serial No. DBS 307
1. Control Activities
2. Control Test
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Limulus Testing for Pyrogens

Previous Serial Number: None

Principal Investigator: H. Donald Hochstein, Dr. P.H.

Other Investigators: Edward B. Seligmann Jr., Ph.D.
James F. Cooper, MPH

Cooperating Units: Bureau of Radiological Health, Food and Drug Administration

Man Years

Total: 0.5
Professional: 0.5
Other: 0

Project Description:

Objectives: To determine the applicability of the Limulus lysate test for detecting pyrogens in both biological and pharmaceutical products.

1) Parallel testing of in vitro and in vivo pyrogen tests.

2) Determine the endpoint for a positive Limulus test.

3) Determine the best method for preparation and storage of the lysate.

Methods Employed: The Limulus test was conducted in parallel with the rabbit pyrogen test. The lysate preparation, storage and endpoint determinations were varied and tested in conjunction with the rabbit assay.

Major Findings: We found that after the lysate was freeze-dried, it could be stored at 5° C. for at least one year without loss of potency. The most reproducible endpoint of the Limulus test was a firm gel following one hour incubation in a 37° C. water bath.

Proposed Course of Project: In the very near future we plan to install salt water holding tanks. This will enable us to bleed the Limulus polyphemus (horseshoe crab) at designated intervals while controlling food and temperature. This will also give us a constant supply of lysate.

Honors and Awards: None

Publications: Cooper, J.F., Hochstein, H.D. and Seligmann, E.B., The Limulus Test for Endotoxin (Pyrogen) in Radiopharmaceuticals and Biologicals. Bull. Parenteral Drug Assoc. 1972 (In Press).

CONTRACT NARRATIVES
DIVISION OF BIOLOGICS STANDARDS
July 1, 1971 through June 30, 1972

STATE OF MICHIGAN, DEPARTMENT OF PUBLIC HEALTH, 3500 N. LOGAN, LANSING, MICHIGAN (NIH-DBS-72-2081)

Title: Development of a Stable Anthrax Vaccine and Improvement of Potency Testing

Contractor's Project Director: Dr. George R. Anderson

Project Officer (DBS): Dr. Edward B. Seligmann Jr.

Objectives: (1) To produce a highly stable anthrax vaccine to serve as a reference preparation. (2) Study the effects of various adsorbants, stabilizers, and preservatives on the safety, stability, and potency of anthrax vaccine. (3) To improve the present potency test in order to reduce variability and increase precision. (4) To investigate the possibility of using an immune serum as a reference in place of a vaccine as a reference.

Methods Employed: a) Prepare both liquid and adsorbed anthrax vaccines to serve as starting materials for the preparation of experimental vaccines containing a variety of stabilizing substances. b) Variables in the potency test will be studied including a study of mice versus guinea pigs and more than one strain of challenge organism. c) Accelerated storage at high temperatures will be used to screen the stablized vaccine.

Major Findings: None

Significance to DBS Program and Bio-Medical Research: It is anticipated that this project will provide DBS with a more stable reference preparation and an improved potency test for anthrax vaccine.

Proposed Course of Project: This project will continue into fiscal year 1973.

Date Contract Initiated: January 1, 1972

Current Annual Level: $23,183

Annual Report of the Laboratory of Pathology
Division of Biologics Standards
July 1, 1971 through June 30, 1972

The program of the Laboratory of Pathology basically has been that of the study of the pathogenesis of infectious diseases and immunologic processes. This research program is presently quite broad and reflects the diversity of interests of the scientific personnel in the laboratory. These interests include cytology, virology, immunology, oncology, histology, and primate biology. All of these fields relate, however, to projects designed to meet the overall needs of the Division in regard to the safety and efficacy of biological products both new and old.

During the past year, several significant changes in the Division have led to a proposed reorganization of the Laboratory of Pathology. First, the Chief of the Laboratory was appointed in October to the position of Assistant Director (Research Contracts) and has since functioned in both capacities. Second, some of the newer activities of the Division can be implemented more efficiently by certain organizational changes in LP. Thus, a reorganization of the laboratory was set up at the end of April, 1972 as follows:

1. Appointment of Stanley H. Singer, M.D. to the newly created position of Assistant Chief, LP. The creation of this position allows greater flexibility of the Chief in performing her duties in both the Laboratory and the Office of the Director.

2. Creation of a Section of Experimental Cytology in place of the Histopathology Section and designation of John C. Petricciani, M.D., as Chief.

3. Creation of a Section of Primate Biology in place of the Animal Test Section. Douglas Lorenz, Ph.D., is designated Acting Chief.

The change of titles of the sections indicates the broadening of the functions and research activities of the sections as well as the Laboratory as a whole and also recognizes the trend of future developmental studies relating to safety and efficacy of biological products. Clearly both cytologic studies (in the broadest use of the term), and studies showing the effects of biologics on a wide variety of subhuman primates are essential for such regulatory functions.

However, the Laboratory, in order to fully carry out its regulatory and research functions still has need of further scientific personnel particularly, as stated last year, another individual with experience in pathology in order to perform the final aspects of the animal safety tests and collaborative studies with other investigators.

Research Activities

Studies of the various candidate agents of human hepatitis have continued with the major emphasis during this year on serum hepatitis or hepatitis Type B. Drs. Chisari, Kirschstein and Lorenz and Mr. McGrath of LP along with Dr. Barker, LVI, and the staff of Hazleton Laboratories have induced evidence of infection in six chimpanzees after inoculation of human serum previously found infectious for man. Infection is usually manifest by the development of circulating hepatitis B antigen (hepatitis associated antigen) 3-5 months after inoculation and/or of circulating antibody. In two animals, evidence of liver damage also was found. Histologic changes of acute viral hepatitis were seen. Thus, an animal model of hepatitis, as a disease entity, has been developed, and expanded studies of the pathogenesis are in progress. (Project No. 314, p.33).

Mr. McGrath has studied liver biopsy specimens from the chimpanzees and has found "virus-like" particles in the nuclei of hepatocytes similar to those seen in human hepatocytes in liver biopsy material obtained from patients with serum hepatitis. (Project No. 317, p. 41).

Drs. Petricciani and Lorenz and Mrs. Hopps, LVI, in collaboration with the work performed under Contract No. NIH-69-100 have continued the studies of the nonhuman primate diploid cell line. The cell line appears to be very promising in regard to the growth of those vaccines from which vaccines are currently being produced or are contemplated in the future. Dr. Petricciani has developed a sensitive system of evaluation of the oncogenic potential of primate cells in cultures using primates treated with specific anti-lymphocyte globulin prior to inoculation with the cell suspensions to be tested. Cultured cells from known human malignancies such as KB cells and HeLa cells form tumors in monkeys so treated whereas neither primary monkey cells or the diploid line will do so. (Project No. 310, p. 18).

Dr. Singer has been studying the respective roles of immunity and interferon production in respiratory infections. Mice treated with immunosuppressive drugs develop a more virulent, lethal infection after instillation of Mycoplasma pulmonis into the nares than do untreated mice. On the other hand, similarly treated mice infected with influenza virus have less illness than untreated animals suggesting that the immune response may enhance certain respiratory infections while suppressing others. (Project No. 313, p. 28).

The role of herpes viruses in the development of neoplasms has been investigated by Drs. Kirschstein and Lorenz in collaboration with Dr. Rabson of NCI. Herpesvirus saimiri, a naturally occurring herpesvirus of squirrel monkeys, causes the development of lymphoma in the marmoset. Evidence of the presence of the virus or the viral genome can only be demonstrated by sensitive cocultivation techniques. (Project No. 44, p. 9).

Dr. Shabo has applied the hydrogen peroxidase labeling technique to the study of viral antigens in cell culture and in animals (Project No. 202, p. 30).

The laboratory staff participate in a large program of collaborative research projects which are underway not only with other laboratories in the Division but with NCI, NIAID, NINDS, and NIMH. Some of these projects are specifically related to control activities of the Division and others have a tangential relationship. This collaborative research program as all other research activities is under the direction, supervision, and coordination of the Chief of the Laboratory.

Collaborative (Contracts) Activities

The Laboratory of Pathology plays a very active role in the collaborative program of the Division, since most if not all contracts have aspects relating to pathogenesis and require pathologic interpretation. The Chief of the Laboratory of Pathology in the past, worked very closely with the various project officers, the Contracts Operations Officer, and the Director, DBS in helping to plan and execute various projects and to review progress reports of those contracts already in existence. She also reviews histologic material from contract work as necessary. In order to formalize this relationship, the Chief of LP was appointed Assistant Director (Research Contracts).

Dr. Petricciani has served as Project Officer for four contracts concerning the development of diploid cell strains from subhuman primates and another contract concerning survival of donor white blood cells in recipients. He also served as an Assistant Project Officer for one contract while Dr. Lorenz served as Assistant Project Officer for three contracts. Dr. Chisari serves as Assistant Project Officer for one contract. Dr. Singer serves as project officer on one contract.

Control Activities

During the past year, the Laboratory has performed neurovirulence tests on 20 lots of Poliovirus Vaccine, Live, Oral, for release as well as tested the Reference Vaccine on five occasions. A total of 825 monkeys have been used for this purpose. The safety of these vaccines has been well controlled. Four potency tests on Poliomyelitis Vaccine were performed.

One hundred and fifteen vervet monkeys and 52 rhesus monkeys were sacrificed in order to remove kidneys for the Cell Biology Section, LVR. Three hundred and eleven rabbits were similarly sacrificed.

The Histopathology Section produced 22,525 sections for light microscopy, 2500 sections for electron microscopic study, and 650 scanning electron microscopy negatives. Seven thousand photographic prints were made.

The Chief of the Laboratory has served as Chairman of seven license application review committees and on five other such committees. She has prepared final regulatory standards for Poliovirus Vaccine, Live, Oral; Adenovirus Vaccine, Live, Oral; and L-Asparaginase. Protocols have been reviewed by Dr. Kirschstein and Dr. Singer prior to release as follows: Poliovirus Vaccine, Live, Oral: 102; Poliomyelitis Vaccine: 5; Live Attenuated Measles Virus Vaccine: 6; Mumps Virus Vaccine, Live: 7; Rubella Virus Vaccine: 5. Each permanent staff member has participated in the annual inspection program performing inspections at five establishments. Numerous Investigational New Drug Applications were reviewed.

Other Activities and Honors:

Dr. Ruth L. Kirschstein:	Appointed Assistant Director (Research Contracts) DBS, October, 1971.
	Superior Service Award of the Department of Health, Education and Welfare, June, 1971.
	Appointed to the Primate Research Center's Advisory Committee, Division of Research Resources, NIH, 1972.
	Appointed to the Board of Consultants, Journal of Medical Primatology.
	Invited speaker at the Oholo Biological Conference of Israel, March, 1972.
	Member of Ad Hoc Committee on Anti-Viral Substances, NIAID.
Dr. Stanley H. Singer:	Appointed Assistant Laboratory Chief, LP
	Invited participant - International Colloquium on Interferon and Interferon Inducers, Leuven, Belgium, September, 1971.
	Member Ad Hoc Committee on Anti-Viral Substances, NIAID.
Dr. John Petricciani:	Appointed Chief, Experimental Cytology Section, LP.
	Guest Lecturer, Duke University Department of Biochemistry, March, 1972.
	Guest Lecturer, Nevada State Medical Society, 1972.

	Contributor, 1973 edition of "Science and Technology."
Dr. Douglas Lorenz:	Appointed Acting Chief, Primate Biology Section, LP.

Serial No. DBS 41
1. Pathology
2. Animal Testing and Histopathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Neurovirulence Testing on Poliovirus Vaccine, Live, Oral, in Monkeys

Previous Serial Number: 41

Principal Investigator: Ruth L. Kirschstein, M.D.

Other Investigators: Douglas Lorenz, Ph.D., LP-DBS
Alan L. Shabo, M.D., LP-DBS
Francis V. Chisari, M.D., LP-DBS
George Rusten, LP-DBS
Robert Gray, LP-DBS

Cooperating Units: Serology Section, LVI, screens monkey serums for freedom from antibody to poliovirus

Man Years:
Total: 5.0
Professional: 1.0
Other: 4.0

Project Description:

Objectives: To develop information concerning the reproducibility and sensitivity of neurovirulence tests of Live Poliovirus Vaccines, to refine techniques used in the test to assure safety of the vaccine, to use new test animal systems, and to test the vaccines being produced on new cell substrates.

Methods Employed: Monkeys were inoculated with vaccine or dilutions thereof either intrathalamically, intraspinally, or intramuscularly and observed for evidence of developing paralysis over a period of 17-18 days. The monkeys were then sacrificed and the central nervous system examined histologically for evidence of poliomyelitis. In addition, tissues were saved for virus isolation attempts. These studies were performed as outlined in the PHS Regulations for Poliovirus Vaccine, Live, Oral.

Major Findings: Poliovirus Vaccine, Live, Oral produced on a human diploid cell substrate was licensed for use on March 3, 1972. This approval was based on laboratory and clinical studies performed by the manufacturer and LP-DBS so as to assure that this vaccine was equal to the already available vaccine produced on monkey kidney cell cultures.

Twenty lots of the new vaccine were tested including the five consecutively produced lots of each of the three types of poliovirus, Type I, Type II, and Type III. Such tests indicated that the neurovirulence properties of the vaccine which are the most important criteria of attenuation have not been altered by the use of this new substrate.

In order generally to ensure the safety of Poliovirus Vaccine, Live, Oral, licensed for use in the United States, all lots of vaccine submitted for release have been tested. Eight lots of Type 3 and 12 of Type 1 have been tested as well as 1 lot of Type 2 vaccine. The Reference Vaccine has been tested six times.

In order to have primates always available, experimental testing in cynomologus monkeys is underway.

Significance to Biomedical Research and the Program of the Division: These studies are designed to assure standardization of neurovirulence testing of poliovirus vaccines throughout the world, to assure the safety of such vaccines, and to improve testing procedures.

Proposed Course of Project: It is anticipated that collaborative studies to standardize safety testing of vaccines will be a continuing activity and that, as in the past, scientists from all over the world will be sent to learn these essential techniques.

Honors and Awards: Dr. Ruth L. Kirschstein - Superior Service Award of the Department of Health, Education and Welfare, June, 1971.

Publications:

Kirschstein, R. L., and Palmer, A. E., A Primate Program Developed for the Testing of Biological Products. Medical Primatology, pp. 886-894, S. Karger and Co., Basel, Switzerland, 1970.

Serial No. DBS 44
1. Pathology
2. Animal Testing and Histopathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: The Pathogenesis of Infectious Diseases

Previous Serial Number: 44

Principal Investigator: Ruth L. Kirschstein, M.D.

Other Investigators: Francis Chisari, M.D., LP-DBS
Alan Rabson, M.D., NCI
Elizabeth Peters, LP-DBS
Stanley Singer, M.D., LP-DBS
Philip McGrath, LP-DBS
George Rusten, LP-DBS
Michael Barile, Ph.D., LBBP-DBS
Pinya Cohen, Ph.D., LBBP-DBS
Carolyn Hardegree, M.D., LBP-DBS
Lewellys F. Barker, M.D., LVI-DBS
Douglas Lorenz, Ph.D., LP-DBS
Henry Orr, Ph.D., LVR-DBS

Cooperating Units: Pathologic Anatomy Branch, NCI
Laboratory of Bacterial Products, DBS
Laboratory of Blood and Blood Products, DBS
Laboratory of Viral Immunology, DBS
Laboratory of Virology and Rickettsiology, DBS

Man Years:
Total: 7.0
Professional: 1.8
Other: 5.2

Project Description:

Objectives: Experimental infectious diseases are studied as to pathogenesis in the hope that this may further elucidate the pathogenesis of infectious diseases in man and lead to their prevention.

Methods Employed: Infectious agents and related substances or substances causing an immunologic response are injected into a variety of animals when newborn or adult, and gross autopsies and histologic examinations are performed at timed intervals so that the development of the disease process and the pathologic changes may be studied.

Major Findings: (1) (With Drs. Rabson, Lorenz and Mrs. Peters) The pathogenesis of the Herpes group of viruses in newborn and adult animals after various routes of inoculation has been studied. One virus, Herpesvirus saimiri induces a lymphoma in marmosets similar to lymphoma in man. Cell cultures of malignant lymphoid cells do not yield virus unless sensitive cocultivation techniques are used.

(2) (With Dr. Rabson and Mr. Rusten) An oral estrogen-progesterone compound has been fed to rhesus monkeys in an attempt to unmask any latent mammary tissue virus. Although no virus has been found, one out of six monkeys developed a typical mammary carcinoma with metastases which caused the death of the animal.

(3) (With Dr. Cohen and Dr. Hardegree) Studies of the tumors occurring in mice after subcutaneous inoculation of components of oil adjuvant, either alone, in combination with other components or in combination with carcinogens or of other adjuvant substances are continuing as a part of the evaluation of the results obtained in the study carried out in Contract No. PH43-67-679.

(4) (With Dr. Singer and Dr. Barile) Studies of the interaction of cells and viruses as well as cells, viruses, and mycoplasma are continuing as described in Project No. 201.

(5) (With Dr. Singer) Studies of interferon action during respiratory infection in comparison to the role of immunity are in progress and are described in Project No. 313.

(6) (With Drs. Lorenz, Chisari, Barker, and Mr. McGrath) Studies of candidate agents of human infectious and serum hepatitis in various species of primates including chimpanzees are being expanded. The work is being done as described in Project Numbers 133 and 317 and Contract No. NIH-71-2040.

(7) (With Dr. Orr) Histologic identification of tumor tissue used to establish cell cultures is made.

Significance to Biomedical Research and the Program of the Division: These studies are designed to elucidate aspects of the pathogenesis of infectious diseases in animals so that these models may serve to help provide the means of prevention of such diseases in man.

Proposed Course of Project: It is expected that this program will expand in 1972-73, particularly as the demonstration of efficacy of biological products must be related to the pathogenesis of the diseases such products prevent.

Honors and Awards: Dr. Ruth L. Kirschstein continues to serve as a member of Ad Hoc Committee on Anti-Viral Substances, NIAID.

Publications:

Rabson, Alan S., O'Conor, G. T., Lorenz, D. E., Kirschstein, R. L., Legallais, F. Y., and Tralka, T. S.: Lymphoid cell-culture line derived from lymph node of marmoset infected with Herpesvirus saimiri--Preliminary Report. J. Nat. Cancer Inst. 46:1099-1109, 1971.

Orr, Henry C., Harris, L. E., Bader, A. V., Kirschstein, R. L., and Probst, P. G.: Cultivation of Cells From a Fibroma in a Rattlesnake, Crotalus horridus. J. Nat. Cancer Inst. 48:259-264, 1972.

Kirschstein, R. L., Rabson, A. S., and Rusten, G., Infiltrating Duct Carcinoma of the Mammary Gland of a Rhesus Monkey after Administration of an Oral Contraceptive: A Preliminary Report, J. Nat. Cancer Inst. 48: 551-556, 1972.

Singer, S. H., Ford, M., Barile, M., and Kirschstein, R. L., Effect of Mycoplasmas on Virus Replication and Plaque Formations in Mouse Cells, Proc. Soc. Exp. Biol. and Med. 139:56-58, 1972.

Singer, S. H., Ford, M., and Kirschstein, R. L., Respiratory Diseases In Cyclophosphamide Treated Mice. I. Increased Virulence of Mycoplasma pulmonis. Infection and Immunity (In press).

Singer, S. H., Noguchi, P., and Kirschstein, R. L., Respiratory Diseases In Cyclophosphamide Treated Mice. II. Decreased Virulence of PR8 Influenza Virus. Infection and Immunity (In press).

Serial No. DBS 112
1. Pathology
2. Animal Testing and Histopathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Developmental Studies and Control Testing of Attenuated Live Virus Vaccines

Previous Serial Number: 112

Principal Investigator: Ruth L. Kirschstein, M.D.

Other Investigators: Alan L. Shabo, M.D., LP-DBS
Nicola Tauraso, M.D., LVR-DBS
Edward B. Seligmann, Ph.D., LCA-DBS
Paul Albrecht, M.D., LVR-DBS
Douglas Lorenz, Ph.D., LP-DBS

Cooperating Units: Laboratory of Virology and Rickettsiology, DBS
Laboratory of Control Activities, DBS

Man Years:
Total: 2.5
Professional: 0.4
Other: 2.1

Project Description:

Objectives: To gain information concerning the pathogenesis of viral encephalitides in monkeys so that safety tests for attenuated live virus vaccines can be meaningful and the safety of the vaccines assured.

Methods Employed: Rhesus monkeys are inoculated with both wild and attenuated virus preparations, using various routes of administration depending on the mode of action of the particular virus. Animals are observed for evidence of disease and specimens of blood and various tissues are taken for evidence of viral replication. At the end of the observation period which depends on the virus and the route of inoculation, all animals are bled to determine antibody levels. The monkeys are then sacrificed and various organs are examined histologically and assayed for presence of virus.

Major Findings: (1) (With LVR and LCA) Studies of Yellow Fever Vaccine (17D strain) after removal of the contaminating avian leukosis virus indicate that its neurovirulence has not been altered and that it is immunogenic for monkeys. This indicates that the vaccine has retained its desirable properties during the processing to remove the adventitious virus. Studies of other strains, particularly cell culture adapted strains, are underway.

(2) (With LVR) Attempts to adapt human measles virus to nonimmune primate hosts are continuing. Differences in pathogenesis of various strains have been demonstrated as described in Project No. 202.

(3) Based on extensive experience over the past 10 years, it has been determined that once the measles, mumps, and rubella virus strains have been shown to be non-encephalitogenic and free of adventitious agents, routine neurovirulence tests on all lots of rubella, measles, and mumps vaccine need not be performed. Regulations to this effect will be adopted shortly.

Significance to Biomedical Research and the Program of the Division: These studies are designed to develop information which allow control testing of new viral vaccines as well as some of the older vaccines to be more meaningful. Such tests will only be required if they assure increased safety of the products.

Proposed Course of Project: It is expected that this program will continue at its present level.

Honors and Awards: None

Publications:

Schumacher, H. P., Albrecht, P., Clark, R. G., Kirschstein, R. L., and Tauraso, N. M., Intracerebral Inoculation of Rhesus Monkeys with a Strain of Measles Virus Isolated from a Case of Subacute Sclerosing Panencephalitis. Infection and Immunity 4:419-424, 1971.

Serial No. DBS 133
1. Pathology
2. Animal Testing and Histopathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Studies of Hepatitis in Subhuman Primates and of Marmosets

Previous Serial Number: 133

Principal Investigator: Douglas Lorenz, Ph.D., LP-DBS

Other Investigators: Ruth L. Kirschstein, M.D., LP-DBS
Francis Chisari, M.D., LP-DBS
Richard Washington, LP-DBS
Elizabeth Peters, LP-DBS
Lewellys Barker, M.D., LVI-DBS

Cooperating Units: Laboratory of Viral Immunology, DBS

Man Years:
Total: 3.5
Professional: 1.5
Other: 2.0

Project Description:

Objectives: (1) To maintain marmosets under laboratory conditions, (2) to describe the microbiology of these animals, and (3) to use marmosets, as well as other subhuman primates, to study human hepatitis.

Methods Employed: Old World monkeys are maintained by standard procedures common to NIH for over a decade. New World monkeys are housed in separate rooms. Two months of quarantine are conducted by the Laboratory of Pathology. Wayne Monkey Chow is provided instead of the NIH monkey chow because of the increased protein. Classical hematological and bacteriological methods are employed. Blood chemistry determinations are conducted under contract. Determination of Hepatitis B antigen and antibody are conducted by Dr. Barker's laboratory.

Major Findings: (1) Colony maintenance: The group of marmosets, Saguinus mystax which has been held 3 years to evaluate maintenance and breeding has decreased to 28 because 3 breeding females died when they were unable to deliver. Each animal was carrying 3 fetuses. The vitality of this colony is otherwise excellent.

By comparison, only 30 percent of a new group of Saguinus mystax survived the quarantine period and preliminary bleedings and biopsies. These animals were received directly from Peru. Twenty-five percent of the S. nigricollis survived quarantine and preliminary work up. These animals were held at least 30 days in Florida before being sent to the laboratory.

(2) Hepatitis Studies: Nineteen Macaca speciosa (stump-tail monkeys) of various ages were inoculated with sera containing Hepatitis B virus. The animals were examined weekly for 16 weeks and monthly for an additional 2 months for changes in serum transaminase levels, viral antigen, and viral antibody. Liver biopsies were performed only in the early weeks of the experiments. Small amounts of antibody to the virus were detected by Dr. Barker's group several weeks after the animals were inoculated, but not enough nor of sufficient duration to indicate an infection. Thus stump-tail monkeys were eliminated as an experimental animal for Hepatitis B studies.

Newborn Macaca mulatta, M. nemestrina, and Cercopithecus aethiops were also inoculated and examined on a schedule similar to that described in the preceding paragraph. Sufficient viral antibody was found in the M. mulatta to indicate an active infection. These results confirmed a report in press by another group of workers. M. mulatta is now considered an experimental animal for Hepatitis B.

An experiment is in progress to determine the titer of two standard Hepatitis B pools using adolescent M. mulatta. The experiment will continue for 6 months.

Two other long-term experiments are in progress in which two species of marmosets, Saguinus nigricollis and S. mystax are inoculated with material expected to contain Hepatitis A virus. In these experiments, serum transaminase levels and liver histology are evaluated weekly for signs of disease. Serological determinations are not available. These experiments should help us confirm the findings that marmosets are susceptible to Hepatitis A virus; however, the problems of adapting these animals to laboratory conditions are very great.

Significance to Biomedical Research and the Program of the Division: The work contributes to the development of experimental animal models for human hepatitis and, thus, to eventual studies of prevention of this disease.

Proposed Course of Project: The major effort will be directed toward hepatitis experiments in primates. Hepatitis A will be studied in marmosets; Hepatitis B will be studied in rhesus monkeys.

Honors and Awards: None

Publications:

Petricciani, J., H. Hopps, and D. Lorenz, 1971, Subhuman Primate Diploid Cells: Possible Substrates for Production of Virus Vaccines. Science 174:1025-1027, 1971.

Rabson, A., G. O'Conor, D. Lorenz, R. Kirschstein, F. Legallais, and T. Tralka, Lymphoid Cell - Culture Line Derived from Lymph Node of Marmoset Infected with Herpesvirus saimiri - Preliminary Report. J. Nat. Cancer Inst. 46:1099-1109, 1971.

Serial No. DBS 162
1. Pathology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: The Effect of Bacterial Viruses on Animal Cells

Previous Serial Number: 162

Principal Investigator: John C. Petricciani, M.D.

Other Investigators: Carl R. Merril, M.D.

Cooperating Units: Laboratory of General and Comparative Biochemistry, NIMH

Man Years:
Total: 2.00
Professional: 2.00
Other: 0

Project Description:

Objectives: The uptake and subsequent fate of bacteriophage in mammalian cell cultures is being studied.

Methods Employed: Both intact phage and extracted nucleic acid are inoculated onto mammalian cell cultures which are then observed for morphologic and biochemical changes.

Major Findings: Bacterial genes appear to be biologically active in mammalian cells in culture, and bacteriophage can be used as a vehicle to transport such genes into the cells.

Significance to Biomedical Research and the Program of the Division: These studies may provide information on the possible use of bacterial viruses and bacterial genes as agents in genetic therapy.

Proposed Course of Project: These studies have been terminated.

Honors and Awards: Articles describing this research appeared in most major United States and foreign newspapers in October 1971. Invited as

guest lecturer: (1) Duke University Department of Biochemistry (March 1972); (2) Nevada State Medical Society Scientific Session (November 1972); Invited to write an article for the 1973 edition of "Science and Technology."

Publications:

Merril, C. R., Geier, M. R., and Petricciani, J. C.: Bacterial virus gene expression in human cells. Nature 233:398-400, 1971.

Petricciani, J. C., Binder, M. K., Merril, C. R., and Geier, M. R.: Galactose utilization in galactosemia. Science 175:1368-1370, 1972.

Binder, M. K., Petricciani, J. C., Merril, C. R., and Geier, M. R.: Aspects of galactose metabolism in normal and galactosemic cell cultures. Med. Annal. Distr. Columbia (in press).

Serial No. DBS 310
1. Pathology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Development of Improved in vivo Assays for Cell Tumorigenicity

Previous Serial Number: None

Principal Investigator: John C. Petricciani, M.D., LP-DBS

Other Investigators: Ruth L. Kirschstein, M.D., LP-DBS

Cooperating Units: None

Man Years:
Total: 0.30
Professional: 0.20
Other: 0.10

Project Description:

Objectives: To develop more sensitive assays for cell tumorigenicity in the hamster and in the subhuman primate.

Methods Employed: Newborn hamsters, and newborn, infant, and juvenile monkeys (Macaca mulatta and Cercopithecus aethiops) are treated with antilymphocyte serum or globulin and cells are inoculated in vivo.

Major Findings: A more sensitive hamster assay and a new primate assay for cell tumorigenicity have been established. Each of these test systems discriminates between cells known to be normal and those known to have been derived from neoplasms such as KB cells and HeLa cells.

Significance to Biomedical Research and the Program of the Division: These assay systems allow a greater assurance that cells used in vaccine production do not have tumorigenic properties.

Proposed Course of Project: Refinements in technique and additional cell testing will continue in the new year.

Honors and Awards: None

Publications:

Wallace, R., Vasington, P. J., and Petricciani, J. C.: Heterotransplantation of cultured cell lines in newborn hamsters treated with antilymphocyte serum. Nature 230:454-455, 1971.

Petricciani, J. C., Kirschstein, R. L., Wallace, R., and Martin, D., Assay for cell tumorigenicity in subhuman primates treated with antilymphocyte globulin, J. Nat. Cancer Inst. 48:705, 1972.

Serial No. DBS 311
1. Pathology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Studies on Cells Now Used or Considered for Use in Virus Vaccine Production

Previous Serial Number: None

Principal Investigator: John C. Petricciani, M.D.

Other Investigators: Douglas Lorenz, Ph. D., LP-DBS
Hope Hopps, LVI-DBS

Cooperating Units: Laboratory of Viral Immunology, DBS

Man Years:
Total: 0.1
Professional: 0.1
Other: None

Project Description:

Objectives: To establish various in vitro characteristics of cells currently used or considered for use in virus vaccine production.

Methods Employed: Cell cultures are examined with respect to senescense, abberant subpopulations, in vivo tumorigenicity, and hexose metabolism.

Major Findings: Subhuman primate diploid cell lines can be established, and at least one such line appears acceptable as a candidate for use in vaccine production.

Significance to Bio-Medical Research and the Program of the Division: The evaluation of cell substrates for virus vaccine production is necessary in order to establish characteristics which may be useful as markers or which may eventually serve as standards for acceptability.

Proposed Course of Project: This study will continue into the coming year.

Honors and Awards: None

Publications:

Petricciani, J. C., Hopps, H. E., Lorenz, D. E.: Subhuman Primate Diploid Cells: Possible Substrates for Production of Virus Vaccines. Science 174:1025-1027, 1971.

Serial No. DBS 200
1. Pathology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Interferon Production by Allergenic Products

Previous Serial Number: 200

Principal Investigators: Stanley H. Singer, M.D.
M. Carolyn Hardegree, M.D.

Other Investigators: Peter J. Gerone, Sc.D.

Cooperating Units: Laboratory of Bacterial Products, DBS
Virus and Rickettsia Division, Ft. Detrick

Man Years:
Total: 0.2
Professional: 0.1
Other: 0.1

Project Description:

Objectives: (1) To determine whether or not bacterial vaccines with "No U. S. Standard of Potency" and allergenic extracts are capable of inducing interferon.

(2) To determine whether these agents can protect against respiratory infections in experimental animals.

Methods Employed: Mice are injected by various routes with the above described biologics. Interferon titers from pooled sera are determined using standard virus yield reduction assays in primary mouse embryo cell cultures. The antiviral activity achieved in mice is determined by a live virus challenge using a variety of routes.

Major Findings: High levels of circulating interferon are induced by certain of the bacterial vaccines and allergenic extracts and interferon production has been shown after inoculation by a variety of routes. Following challenge with two respiratory viruses the following was noted: a) a single intravenous injection of allergenic extract of house dust, but not bacterial vaccine, decreased the amount of parainfluenza type 1 virus recovered from the lungs of treated animals but had no effect on recovery

(155)

of influenza virus; b) Multiple, but not a single, subcutaneous injections of house dust extract significantly decreased the role of infectivity with parainfluenza virus but not influenza virus.

Significance to Biomedical Research and the Program of the Division: It has been determined that certain licensed biologics have the capacity to induce interferon in mice. Protection against certain respiratory viral challenges under defined experimental conditions has been shown. It is possible that this activity may be associated with the clinical effectiveness of this type of product in man. The information will determine the optimal conditions for use of these products in man and their possible efficacy.

Proposed Course of Project: These studies will continue during the next fiscal year and be expanded to include a study of the efficacy of these products against direct respiratory challenge with pathogenic bacteria.

Honors and Awards: None

Publications:

Singer, S. H. and Hardegree, M. C. Induction of Interferon by Bacterial Vaccines and Allergenic Extracts. Journal of Allergy 47:332-340, 1971.

Serial No. DBS 201
1. Pathology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Mycoplasma-Virus Interaction

Previous Serial Number: 201

Principal Investigator: Stanley H. Singer, M.D.

Other Investigators: Ruth L. Kirschstein, M.D., LP-DBS
Michael Barile, Ph.D., LBP-DBS

Cooperating Units: Laboratory of Bacterial Products, DBS

Man Years:
Total: 0.6
Professional: 0.3
Other: 0.3

Project Description:

Objectives: The effects of dual infection with a mycoplasma and a virus are being studied in in vitro and in vivo situations.

Methods Employed: Chronic mycoplasma infection is established and then cultures or animals are super-infected with viruses. Adsorption, penetration, replication, interferon production, and pathogenicity of the virus are then determined.

Major Findings: We have previously demonstrated that contamination of hamster cell cultures by mycoplasma can influence interferon production and, thus, virus production. We have extended these findings in vitro to other species using both continuous cell lines and primary cell cultures. We have demonstrated also that the final result of virus multiplication is not only a function of the particular mycoplasma, the particular virus, and the particular cell species used, but also depends on whether the cell culture is primary or continuous. In addition, we have demonstrated that mycoplasma contamination of cell cultures can modify (decrease) their interferon response after exposure to interferon inducers.

Significance to Biomedical Research and the Program of the Division: Mycoplasmas are found as contaminants in many cell culture systems. The above work has illustrated that they may influence the results of any tests

performed on such cells. This is of importance not only in experimental procedures, but also in any standardization tests utilizing cell cultures. Also, since mycoplasma can be isolated from a substantial proportion of the population, all organs from each animal are examined histologically for evidence of abnormality.

Proposed Course of Project: These studies will be combined with those listed under Project No. 313.

Honors and Awards: None

Publications:

Singer, S. H., Ford, M., Barile, M., and Kirschstein, R. L., Effect of Mycoplasmas on Virus Replication and Plaque Formation in Mouse Cells, Proc. Soc. Exp. Biol. and Med. 139:56-58, 1972.

Singer, S. H. and Ford, M., The Influence of Mycoplasma Infection on the Induction of the Anti-Viral State by Polyinosinic:Polycytidilic Acid. Proc. Soc. Exp. Biol. and Med. 139:1413-1416, 1972.

Serial No. DBS 312
1. Pathology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Interferon Studies

Previous Serial Number: None

Principal Investigators: Stanley H. Singer, M.D.
Marshall Ford

Man Years:
Total: 0.6
Professional: 0.3
Other: 0.3

Project Description:

Objectives: (1) To study the production of interferon during natural infections or after exposure to specific interferon inducers. (2) To evaluate the role of interferon as a possible therapeutic agent. (3) To standardize current procedures designed to measure interferon.

Methods Employed: Interferon is induced in experimental animals by a variety of means. Viral infections are also induced and the role of interferon in modifying these infections is evaluated. Various techniques including plaque reduction, yield reduction, use of different cell substrates, and others, are used to compare interferon assays.

Major Findings: Following interferon induction, test animals enter a hyporesponsive state in that their response to subsequent interferon inducers is much less than the initial response. It was found that this hyporesponsive state is a relative one and that certain organ systems are still capable of yielding a primary type interferon response during this period.

Significance to Biomedical Research and the Program of the Division: There has been recent interest in providing commercially available interferons and interferon inducers. The above studies should add to our knowledge of how much, when, and where interferon could be best utilized.

Proposed Course of Project: These studies will continue in the coming year.

Honors and Awards: Dr. Stanley H. Singer - Invited participant, International Colloquium on Interferon and Interferon Inducers, Leuven, Belgium, September 1971.

Dr. Stanley H. Singer serves as a member, Ad Hoc Committee on Anti-Viral Substances, NIAID.

Publications:

None

Serial No. DBS 313
1. Pathology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Roles of Immunity and Interferon Production in Respiratory Infections

Previous Serial Number: None

Principal Investigator: Stanley H. Singer, M.D., LP-DBS

Other Investigators: Ruth L. Kirschstein, M.D., LP-DBS
Marshall Ford, LP-DBS
Philip Noguchi, LP-DBS

Man Years:
Total: 0.6
Professional: 0.3
Other: 0.3

Project Description:

Objectives: To investigate the role which immunity plays in respiratory illnesses caused by different types of respiratory pathogens.

Methods Employed: Immunosuppressive drugs are administered to test animals which are then infected via the respiratory tract with various pathogens. The course of infection is then studied.

Major Findings: In immunosuppressed animals infected with mycoplasma a chronic smouldering infection becomes a rapidly virulent one. There is early dissemination of the organism to organs other than the lung and higher titers are found. In immunosuppressed animals infected with the PR8 strain of influenza virus the opposite is found, in that immunosuppression delays the appearance of disease despite higher virus titers, less antibody, and less interferon in the immunosuppressed group.

Significance to Biomedical Research and the Program of the Division: An understanding of the role of immune processes in infectious respiratory diseases should be helpful in evaluating vaccines designed to boast immunity to control these diseases.

Proposed Course of Project: These studies will continue in the coming year.

Honors and Awards: None

Publications:

Singer, S. H., Ford, M., and Kirschstein, R. L., Respiratory Diseases in Cyclophosphamide Treated Mice. I. Increased Virulence of Mycoplasma pulmonis. Infection and Immunity (in press).

Singer, S. H., Noguchi, P., and Kirschstein, R. L., Respiratory Diseases in Cyclophosphamide Treated Mice. II. Decreased Virulence of PR8 Influenza Virus. Infection and Immunity (in press).

Serial No. DBS 202
1. Pathology
2. Animal Testing
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Microanatomic Studies on the Central Nervous System, Eye, and of Cell Cultures Under Normal and Pathologic Conditions

Previous Serial Number: 202

Principal Investigator: Alan L. Shabo, M.D.

Other Investigators: Paul Albrecht, M.D., LVR-DBS
Milton Brightman, Ph. D., LNNS-NINDS
Thomas Reese, M.D., LNNS-NINDS
John Petricciani, M.D., LP-DBS
Ruth Kirschstein, M.D., LP-DBS
David Maxwell, M.D., Dept. of Anatomy, U.C.L.A. School of Medicine

Cooperating Units: Dept. of Anatomy, U.C.L.A. School of Medicine
Laboratory of Neuropathology and Neuroanatomical Sciences, NINDS
Laboratory of Virology and Rickettsiology, DBS

Man Years:
Total: 1.75
Professional: 1.0
Other: 0.75

Project Description:

Objectives: To clarify the morphologic substrate of the cerebrospinal fluid (CSF) and aqueous humor pathways under normal and experimentally-induced pathologic conditions in order to define possible membrane barriers.

Methods Employed: Animal tissues are studied by light and electron microscopy with special preparative techniques. In experimental animals, the localization and fate of tracer substances such as peroxidase are defined following systemic or direct injection. A method to couple antibody to peroxidase has been employed successfully to localize intracellular viral antigens.

Major Findings: (1) (With Dr. Maxwell) The arachnoid membrane and blood vessels within the subarachnoid space possess specialized tight junctions that prevent peroxidase passage. However, the pial membrane is permeable to peroxidase which can enter the brain extracellular space from the subarachnoid CSF.

(2) (With Dr. Maxwell) The arachnoid villus of the CSF pathway and the trabecular meshwork of the eye are similar in structure and function as biologic filters that remove substances such as peroxidase from their respective fluids. The endothelium of the arachnoid villus and of Schlemm's canal are intact membranes that partition the vessel lumina from the subarachnoid space and the anterior chamber.

(3) (With Dr. Maxwell) Red blood cells introduced into the aqueous humor become entrapped in the trabecular meshwork where they are degraded by in situ histiocytes. No direct passage or open communication exists between the anterior chamber and Schlemm's canal as has been interpreted using other techniques (i.e. radioautography).

(4) (With Dr. Maxwell) The ciliary process of the eye is similar in structure to the choroid plexus. Between non-pigmented epithelial cells, tight junctions prevent peroxidase passage from the blood into the aqueous humor. These junctions probably represent the site of the blood-aqueous barrier to protein and other large molecules.

(5) (With Drs. Brightman and Reese) The endothelial cell junctions of the arachnoid villus are short, tight junctions which appear to prevent peroxidase passage. However, tilt stage high resolution analysis of serial sections revealed that these junctions are bisected by a narrow cleft that may be peroxidase permeable.

(6) (With Dr. Albrecht) Hamster neurotropic measles virus, after intracerebral inoculation, produces a progressive encephalitis in monkeys that is potentiated by immunosuppression. The Edmonston and Schwarz strains of virus are not encephalitogenic under the same conditions.

(7) (With Drs. Petricciani and Kirschstein) Peroxidase can be conjugated to antibody and used to identify intracellular viral antigens with both the light and electron microscopes. This technique has many advantages over other methods including simpler preparation of reagents, better penetration, and enzymatic amplification of the visible marker.

Significance to Bio-Medical Research and the Program of the Division: These studies provide information that is useful in interpreting normal and pathologic conditions of the CNS and eye. They relate to the pathways through which blood components, biological products, drugs and microorganisms may enter and leave these specialized areas and to the nature of their inherent cellular responses to the presence of foreign substances.

Proposed Course of Project: The appearance and significance of tissue barriers requires redefinition with other tracer substances with special emphasis on their immunologic implications.

Honors and Awards: None

Publications:

Shabo, A. L. and Maxwell, D. S. The subarachnoid space following the introduction of foreign protein: a light and electron microscopic study with peroxidase. J. Neuropath. Exp. Neurol., 30:506-525, 1971.

Shabo, A. L. and Maxwell, D. S. Observations on the fate of blood in the anterior chamber: a light and electron microscopic study of the monkey trabecular meshwork. Am. J. Ophthal., 73:25-36, 1972.

Shabo, A. L. and Brightman, M. B. Permeability to peroxidase of the endothelium in the arachnoid villus of monkeys. Anat. Rec., 172: 404, 1972.

Shabo, A. L. and Maxwell, D. S. The blood-aqueous barrier to tracer protein: a light and electron microscopic study of the primate ciliary process. Microvasc. Res. 4:142-158, 1972.

Shabo, A. L. and Maxwell, D. S. The fine structure of the arachnoid villus under normal and experimental conditions. Acta Neurológica Latino-americana (In press).

Shabo, A. L., Petricciani, J. C., and Kirschstein, R. L. Immuno-peroxidase localization of herpes zoster virus and simian virus 40 in cell culture. App. Microbiol. (In press, May, 1972).

Shabo, A. L. and Maxwell, D. S. The structure of the trabecular meshwork of the primate eye: a light and electron microscopic study of peroxidase. Microvasc. Res. (In press, July, 1972).

Albrecht, P., Shabo, A. L., Burns, G. R., and Tauraso, N. M. Experimental measles encephalitis in normal and cyclophosphamide-suppressed rhesus monkeys. J. Inf. Dis. (In press).

Serial No. DBS 314
1. Pathology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Transmission of Viral Hepatitis Type B to Chimpanzees

Previous Serial Number: None

Principal Investigator: Francis V. Chisari, M.D.

Other Investigators: Lewellys F. Barker, M.D., LVI-DBS
Ruth L. Kirschstein, M.D., LP-DBS
Philip P. McGrath, LP-DBS
Dan W. Dalgard, D.V.M., Hazleton Labs, Vienna, Va.
Thomas S. Edgington, M.D., Scripps Clinic and Research Foundation, La Jolla, California

Cooperating Units: Laboratory of Viral Immunology, DBS
Hazleton Laboratories, Vienna, Virginia
Scripps Clinic and Research Foundation, La Jolla, California

Man Years:
Total: 5
Professional: 2.5
Other: 2.5

Project Description:

Objectives: As an extension of research contract number NIH-71-2040 this project was designed to develop an animal model of viral hepatitis type B which would then facilitate investigation into the pathogenesis and prevention of type B viral hepatitis in humans.

Methods Employed: Six young adult chimpanzees, housed at Hazleton Laboratories, Vienna, Virginia, under research contract were inoculated subcutaneously with human serum known to contain hepatitis B antigen (HB Ag). These animals, and a seventh uninoculated HB Ag carrier, were bled weekly for HB Ag, anti-HB Ag and serum transaminase determinations and complete blood counts. They underwent periodic closed or open liver biopsies and the tissue obtained was studied by means of light and electron microscopy, immunofluorescence microscopy, and tissue culture.

Major Findings: (1) (With Dr. Barker) Inoculation of serum containing HB Ag frequently results in the appearance of detectable circulating HB Ag and/or anti-HB Ag and occasionally results in an elevation of serum transaminase levels.

(2) (With Dr. Kirschstein and Mr. McGrath) Those animals that develop elevated serum transaminase levels also develop histologic changes in their livers characteristic of acute viral hepatitis as seen in man. One of these animals was found to have intranuclear virus-like particles, 22-28 nm in diameter, in its hepatocytes.

(3) (With Dr. Edgington) Frozen liver tissue sections from the chimpanzee which developed light and electron microscopic changes of hepatitis and from the chronic HB Ag carrier (normal liver by light and electron microscopy) demonstrated specific cytoplasmic fluorescence when incubated with fluoresceinated anti-HB Ag.

Significance to Biomedical Research and the Program of the Division: An animal model of viral hepatitis type B is now available. This will enable the future study of the pathogenesis of this type of hepatitis and may assist in the development of a specific vaccine.

Proposed Course of Project: Studies are now underway to evaluate the effect of immunosuppression on the immunologic response to HB Ag and its effects on the development of hepatocellular injury.

Honors and Awards: None

Publications: None

Serial No. DBS 315
1. Pathology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: In Vivo Reversion of Toxicity of Cholera Toxoid

Previous Serial Number: None

Principal Investigator: Francis V. Chisari, M.D.

Other Investigators: Robert S. Northrup, M.D., LBP-DBS

Cooperating Units: Laboratory of Bacterial Products, DBS

Man Years:
Total: 3.5
Professional: 2.0
Other: 1.5

Project Description:

Objectives: To determine the immunogenicity and safety of a formalinized cholera toxoid in rhesus monkeys.

Methods Employed: Thirty adult rhesus (Macaca mulatta) monkeys were inoculated subcutaneously with various formalinized preparations of cholera toxoid and observed daily for local reactions. Venous blood was obtained periodically to determine antitoxic antibody response and to follow serum chemistries.

Major Findings: Cholera toxoid is a potent immunogen producing high serum antitoxin titers after two subcutaneous inoculations of 50 µg at monthly intervals. A severe local reaction develops at the inoculation site which has the appearance and time course of the reaction to cholera toxin and which represents in vivo reversion of toxoid to toxin.

Significance to Biomedical Research and the Program of the Division: This represents part of an effort to develop a more effective vaccine against cholera.

Proposed Course of the Project: Studies are underway to determine what degree of protection is afforded to animals inoculated with new non-reverting toxoids and to elucidate the adjuvant effect of cholera toxin re: its possible desirability as a minor component of new vaccines.

Honors and Awards: None

Publications:

Northrup, R. S., and F. V. Chisari, 1972, Response of Monkeys to Immunization with Cholera Toxoid, Toxin and Vaccine: Reversion of Toxicity of Cholera Toxoid. J. Inf. Dis. (In press).

Serial No. DBS 316
1. Pathology
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Toxicity Studies of L-Asparaginase in Rabbits

Previous Serial Number: None

Principal Investigator: Francis V. Chisari, M.D.

Other Investigators: Ruth L. Kirschstein, M.D., LP-DBS
H. Donald Hochstein, Dr. P.H., LCA-DBS
Edward B. Seligmann, Ph. D., LCA-DBS

Cooperating Units: Laboratory of Control Activities, DBS

Man Years:
Total: 1.0
Professional: 0.5
Other: 0.5

Project Description:

Objectives: L-Asparaginase, a chemotherapeutic agent for acute lymphocytic leukemia of childhood is a biological product subject to the provisions of the Public Health Service Act and thus must be non-pyrogenic. However, in the course of the performance of the pyrogen test, this material was found to be lethal. The objective of this study was to determine the cause of death and the pathogenesis of the lesions caused by L-asparaginase administered intravenously to rabbits in the course of pyrogen testing.

Methods Employed: Fifty-three New Zealand white rabbits were injected intravenously with 1000 international units per kilogram of L-asparaginase obtained from several manufacturers. They were observed hourly until death or for 5 days and periodically bled for serum chemistry determinations. Necropsies were performed on all animals and all tissues were subjected to microscopic examination.

Major Findings: L-asparaginase induced marked cellular necrosis of the parathyroid glands which was associated with severe hypocalcemia, tetany, and death.

Significance to Biomedical Research and the Program of the Division: A model of acute hypocalcemia has been discovered and a potentially dangerous side effect of an agent used in the treatment of human malignancies has been elucidated and characterized.

Proposed Course of Project: The project has been terminated.

Honors and Awards: None

Publications:

Francis V. Chisari, H. Donald Hochstein, Ruth L. Kirschstein, and Edward B. Seligmann, Parathyroid Necrosis and Hypocalcemic Tetany Induced in Rabbits by L-asparaginase, Amer. J. of Path. (Submitted for Publication).

Serial No. DBS 203
1. Pathology
2. Histopathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Scanning and Transmission Electron Microscopy of Materials Used in Testing Biological Products

Previous Serial Number: 203

Principal Investigator: Philip P. McGrath

Other Investigators: John B. Ewell, LP-DBS
Harold Baer, Ph.D., LBP-DBS
Sotiros Chaparas, Ph.D., LBP-DBS
Charles Manclark, Ph.D., LBP-DBS
Lee J. Stevan, M.S., LBP-DBS
Hope E. Hopps, M.S., LVI-DBS
Michael J. Klutch, LVR, DBS
Nicola M. Tauraso, M.D., LVR-DBS

Cooperating Units: Laboratory of Bacterial Products, DBS
Laboratory of Viral Immunology, DBS
Laboratory of Virology and Rickettsiology, DBS

Man Years:
Total: 2.3
Professional: 1.0
Other: 1.3

Project Description:

Objectives: To relate surface and intracellular ultrastructural changes in tissues and cells to infection with microorganisms or response to immunological reagents.

Methods Employed: Samples to be examined are prepared for scanning and transmission electron microscopy. The surface structures of normal samples are compared with experimental samples to determine changes induced by the particular experimental manipulations. These results from the scanning electron microscope are compared with light microscopic and transmission electron microscopic findings and evaluated as to effects of the agents used in the experimental system.

Major Findings: (1) (With Dr. Baer and Mr. Ewell) Studies were initiated to characterize pollen sample used in production of allergenic extracts. All the samples examined contain some foreign matter or pollen but the amount is below 1.0%.

(2) (With Dr. Chaparas) The morphologic characterization of the various species of Mycobacteria may be an aid in reclassifying these organisms. The study will continue until representative examples of most of the major groups of Mycobacteria are examined.

(3) (With Dr. Manclark and Mr. Ewell) Scanning and transmission electron microscopy of intact pertussis bacteria and cell fragments to compare particle morphology with biological activity.

(4) (With Mr. Ewell and Mr. Stevan) Morphological changes of Clostridium Botulium under varying growth conditions as an aid in the study of toxin production.

(5) (With Mrs. Hopps and Mr. Ewell) Cell cultures are being examined in the scanning and transmission electron microscopes to determine cell surface changes which can be correlated with virus growth or contaminant.

(6) (With Mr. Klutch and Dr. Tauraso) Comparative morphology of influenza virus in vaccines and candidate strains for vaccines. In some of the vaccines and the candidate viruses a particle with the morphological characteristic of an adenovirus was seen.

Significance to Bio-Medical Research and the Program of the Division: The scanning electron microscope is a comparatively recent development and its use in bio-medical research has not been exploited. The unique feature of this instrument is its depth of focus which allows cells and organisms to be studied in three dimensions giving a view of surface structure at high magnification unresolvable with any other instrument. These features should help the Division's research and safety testing programs.

Proposed Course of Project: This program will continue with emphasis on biologicals and cell surface interactions.

Honors and Awards: None

Publications:

McGrath, Philip P., Clark, Ronald G., Ewell, John B., and Wehrung, John M., Scanning electron microscopy of the choroid plexus and ventricular wall ependyma in the rhesus monkey, Proc. 29th Ann. Meeting Electron Microscopy Society of America, pp. 270-271, 1971.

Serial No. DBS 317
1. Pathology
2. Histopathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Electron Microscopy of Viral Hepatitis Candidate Agents in Man and Subhuman Primates

Previous Serial Number: None

Principal Investigator: Philip P. McGrath

Other Investigators: Lewellys F. Barker, M.D., LVI-DBS
Ruth L. Kirschstein, M.D., LP-DBS
Francis V. Chisari, M.D., LP-DBS
Douglas Lorenz, Ph.D., LP-DBS
John B. Ewell, LP-DBS
Dan W. Dalgard, D.V.M., Hazleton Laboratories
Vienna, Virginia

Cooperating Units: Laboratory of Viral Immunology, DBS
Hazleton Laboratories, Vienna, Virginia

Man Years:
Total: 2.2
Professional: 1.5
Other: 0.7

Project Description:

Objectives: To study candidate hepatitis virus particles in liver and serum of experimental animals and man as an aid in development of animal models to investigate pathogenesis and prevention of hepatitis in humans.

Methods Employed: Serum samples of human and subhuman primates are examined for possible virus particles by negative staining and immunoelectron microscopy. Thin sections of liver biopsies and necropsies are examined in the electron microscope using heavy metal stains and antibodies tagged with heavy metals. Experimental animals inoculated with known icterogenic sera are followed by sequential serum and liver biopsy samples which are examined for possible virus particles. The electron microscopic findings are correlated with clinical, biochemical, and light microscopic studies performed as described in Project Nos. 133 and 314.

Major Findings: One of six chimpanzees inoculated with Hepatitis B positive serum developed HB Ag and elevated enzyme levels. Intranuclear particles measuring 22-28 nm in diameter were found in some hepatocytes. The appearance of these particles is similar to those seen in human hepatitis B and coincided with the development of HB Ag and elevated serum enzyme levels in this animal. A second type of particle measuring 35 nm in diameter was also seen in the nucleus of this animal; this particle has not been previously reported. Serum from this same animal showed some virus-like particles which have been described previously in sera of humans with Hepatitis B infection.

Significance to Biomedical Research and the Program of the Division: The virus-like particles associated with human hepatitis have been found in the liver cells and serum of an experimentally infected animal. The study of the pathogenesis of this disease in this animal model will help in development of means of prevention of Hepatitis type B.

Proposed Course of Project: Further electron microscopic study of Hepatitis A and B in animal models, tissue culture and serum to further characterize the viruses.

Honors and Awards: None

Publications: None

CONTRACT NARRATIVES
DIVISION OF BIOLOGICS STANDARDS
July 1, 1971 through June 30, 1972

UNIVERSITY OF TEXAS (NIH-70-2247)

Title: Cytological Characterization of Subhuman Primate Cell Cultures

Contractor's Project Director: Dr. T. C. Hsu

Project Officer (DBS): Dr. John C. Petricciani

Objectives: To establish cytogenetic characteristics of primary cell cultures derived from several tissues of the Macaca mulatta and Cercopithecus aethiops species of monkey to serve as guidelines in evaluating cytogenetic data on diploid cell lines of these species.

Methods Employed: Tissues are obtained from animals sacrificed at the DBS for kidney cell cultures and sent to Dr. Hsu's laboratory for preparation of chromosomes and evaluation.

Major Findings: Primary cultures derived from these two species are usually diploid, retain diploid characteristics, and undergo senescence and cell death when serially subcultivated.

Significance to DBS Program and Bio-Medical Research: The data generated in this project will serve as guidelines in evaluating the cytogenetic characteristics of the monkey diploid cells being developed by the DBS for possible use in production of viral vaccines for humans.

Proposed Course of Project: This project will continue into fiscal year 1973 and will include work on rabbit chromosomes.

Date Contract Initiated: Approximately May 1, 1969.

Current Annual Level: $54,395

CONTRACT NARRATIVES
DIVISION OF BIOLOGICS STANDARDS
July 1, 1971 through June 30, 1972

LOS ALAMOS SCIENTIFIC LABORATORIES

Title: Monitoring Ploidy of Cell Cultures by Microfluorometry

Contractor's Project Director: Dr. M. Van Dilla

Project Officer (DBS): Dr. John C. Petricciani

Objectives: To monitor the ploidy of cell cultures by the microfluorometric measurement of the DNA content per cell.

Methods Employed: Model non-biologic systems will be used to refine the resolution of the microfluorometry apparatus now in use, and attempts will be made to detect small subpopulations of cells in cultures provided by DBS based on their different DNA content.

Major Findings: Aneuploid populations of cells have more DNA per cell than normal diploid populations, but the variation in the amount of DNA per cell in the aneuploid cells is no greater than in the diploid cells even though chromosome numbers may vary greatly.

Significance to DBS Program and Bio-Medical Research: The development of a high-speed microfluorometric method for monitoring ploidy offers the possibility of a faster and statistically more accurate technique than those conventionally used to assess the chromosomal stability of cell lines which might be considered for use in vaccine production.

Proposed Course of Project: This project has been terminated.

Date Contract Initiated: February 2, 1970

Current Annual Level: $87,700

CONTRACT NARRATIVES
DIVISION OF BIOLOGICS STANDARDS
July 1, 1971 through June 30, 1972

UNIVERSITY OF PITTSBURGH (PH 43-67-701)

Title: Cytogenetic Follow-up of Intrauterine and Exchange Transfused Children

Contractor's Project Director: Dr. J. Howard Turner

Project Officer (DBS): Dr. John C. Petricciani

Objectives: To continue a prospective cytogenetic study of intrauterine and exchange transfused children by following the persistence of mitotically active transfused cells in the recipients.

Methods Employed: Periodic cytogenetic analyses using peripheral blood will be made on those children already included in the study. Less frequent general pediatric examinations will also be conducted on selected cases.

Major Findings: Persistence of donor leukocytes has been demonstrated in a limited number of children receiving intrauterine and exchange transfusions. A case of apparent in vivo hybridization among donor and recipient leukocytes (lymphocytes) has been detected.

Significance to DBS Program and Bio-Medical Research: The potential for establishing a foreign cell line in the fetus or newborn as the result of a transfusion will continue to be evaluated, and appropriate recommendations might be made to reduce this risk.

Proposed Course of Project: Follow-up of selected cases will continue for several years.

Date Contract Initiated: April 27, 1967

Current Annual Level: $23,773

Publications:

Turner, H. J., Hutchison, D. L., and Petricciani, J. C., Cytogenetic and growth characteristics of human lymphocytes derived from stored donor blood packs. Scand. J. Haemat. 8:169-176, 1971.

CONTRACT NARRATIVES
DIVISION OF BIOLOGICS STANDARDS
July 1, 1971 through June 30, 1972

LEDERLE LABORATORIES (NIH-69-100)

Title: Development of Subhuman Primate Diploid Cell Strains

Contractor's Project Director: Dr. Paul Vasington

Project Officer (DBS): Dr. John C. Petricciani

Objectives: To develop diploid cell lines from fetal tissues of both the Macaca mulatta and Cercopithecus aethiops species of monkey which will be suitable for the production of viral vaccines for human use.

Methods Employed: Fetal tissues from selected pregnant monkeys were used to initiate cell cultures which are being characterized with respect to growth, nutrient requirements, chromosome complement, oncogenic potential, viral susceptibility, and freedom from adventitious agents.

Major Findings: Two new systems to screen cell cultures for tumorigenic potential have been developed. Cell cultures from both species have been propagated to the point of senescence. They retain susceptibility to several viruses, remain diploid, and appear free of adventitious agents. Two cell lines have been distributed to over 40 investigators for independent evaluation. One rhesus cell line continues to appear promising as a candidate for use in vaccine production.

Significance to DBS Program and Bio-Medical Research: The availability of a well-characterized cell system having genetic stability will allow exploration of the potential for vaccine production in cells other than primary cell cultures or human cell strains.

Proposed Course of Project: This project is scheduled for termination in fiscal year 1973.

Date Contract Initiated: Approximately May 1, 1969

Current Annual Level: $103,600

CONTRACT NARRATIVES
DIVISION OF BIOLOGICS STANDARDS
July 1, 1971 through June 30, 1972

MEDICAL COLLEGE OF WISCONSIN (NIH-DBS-72-2088)

Title: Study of Licensed Bacterial Vaccines with no U.S. Standard of Potency

Contractor's Project Director: Dr. Michael W. Rytel

Project Officers (DBS): Dr. Stanley H. Singer
Dr. M. Carolyn Hardegree

Objectives: To determine whether the administration of licensed bacterial vaccines with no U.S. Standard of Potency will induce interferon in man, and whether they will have any effect on the incidence of respiratory infections in these recipients.

Methods Employed: Volunteers will receive immunization with selected licensed bacterial vaccines and will then be monitored for interferon production, and also for the incidence of respiratory infections. An appropriate control group will also be used for comparison.

Major Findings: This contract was recently initiated and there are no results yet available.

Significance to DBS Program and Bio-Medical Research: Selected licensed bacterial vaccines with no U.S. Standard of Potency are commonly used to protect against respiratory infections. They have recently been shown to induce interferon in animal models. This contract will investigate whether they also induce interferon in man and whether they have an effect on protecting against respiratory infections.

Proposed Course of Project: This project will continue during the coming fiscal year.

Date Contract Initiated: March 17, 1972

Current Annual Level: $37,967

CONTRACT NARRATIVES
DIVISION OF BIOLOGICS STANDARDS
July 1, 1971 through June 30, 1972

AMERICAN TYPE CULTURE COLLECTION (NIH-71-2126)

Title: Distribution and Storage of Diploid Animal Cells

Contractor's Project Director: Dr. John Shannon

Project Officer (DBS): Dr. John C. Petricciani

Objectives: To store seed stock and distribution stock ampules of diploid animal cells developed by the DBS, and to distribute ampules of these cells as directed by the DBS.

Methods Employed: Cells in sealed glass ampules are stored in a government-owned liquid nitrogen refrigerator which is continuously monitored for normal operating conditions.

Major Findings: None

Significance to DBS Program and Biomedical Research: This project provides the DBS with a facility having a unique experience in the storage and distribution of cell cultures, and will continue to be used for the several diploid cell lines being developed by the DBS.

Proposed Course of Project: This project will continue into fiscal year 1972.

Date Contract Initiated: May 5, 1971

Current Annual Level: $9,757

CONTRACT NARRATIVES
DIVISION OF BIOLOGICS STANDARDS
July 1, 1971 through June 30, 1972

BIONETICS RESEARCH LABORATORIES (NIH-69-2264)

Title: Maintenance of an African Green Monkey Breeding Colony

Contractor's Project Director: Dr. David P. Martin

Project Officers (DBS): Dr. John C. Petricciani

Objectives: To produce a number of monkey fetuses for use as needed in various projects sponsored by DBS.

Methods Employed: Maintain mature, healthy African green monkeys and mate them by the best proven techniques to obtain fetuses and newborns for DBS Scientists.

Major Findings: The single pair mating technique has continued to be very satisfactory, and the monkeys have proved very useful in several research projects.

Significance to DBS Program and Biomedical Research: These monkeys are being used to provide data on a number of projects where materials and cell lines are being tested for oncogenic potential and/or adventitious agents.

Proposed Course of Project: To be continued.

Date Contract Initiated: June 30, 1967

Current Annual Level: $70,024

Annual Report of the
Laboratory of Virology and Rickettsiology
Division of Biologics Standards

July 1, 1971 through June 30, 1972

A. ADMINISTRATIVE ASPECTS

Introduction.

During the fiscal year, continued emphasis was placed on the development of a program which would coordinate straightforward control with control-related research responsibilities. Progress has been made in phasing out old programs efficiently and developing new meaningful ones.

Mission Statement. The Laboratory encompasses a broad program of research concerning the prevention and treatment of viral and rickettsial infections in man. Specific emphasis is placed upon problems as they relate to the mission of DBS, such as problems concerned with vaccine safety, potency, efficacy and standardization. Constant liaison with the Division's other Laboratories achieves a balance in the harmonious execution of the DBS control and research responsibilities.

Personnel Changes. In June, 1969, the following individuals resigned from the commissioned corps: Martin G. Myers, M.D. who returned as a Resident in Pediatrics at the Johns Hopkins School of Medicine; and Alan Ve Richman, M. D. who became a Resident in Pathology at the University of Pennsylvania School of Medicine. Daniel E. Thor, M.D. received a medical retirement from the commissioned corps in August, 1971; he subsequently assumed the position of Associate Professor of Microbiology, University of Texas Medical School at San Antonio.

Two GS-9 laboratory assistants resigned, one in July, the other in August, 1971. Since these individuals (professional series) were key personnel involved in our influenza vaccine testing program and due to the fact that they were not (could not be) replaced, their loss has seriously hampered our work in this area.

Two personnel (a GS-14 microbiologist and a GS-7 technical assistant) transferred from this to another Division laboratory (LVI).

Additions to the senior professional staff were: (1) James E. Hannah, M.D., who has been assigned a major responsibility to carry out our influenza and yellow fever vaccine volunteer studies; and (2) Mitchell Austin, M.D., who has been assigned to the Viral Pathogenesis Unit, LVR, to work on research problems related to defining pathogenesis of viral infections as these relate to vaccines.

Organizational Changes. The Laboratory of Virology and Rickettsiology (LVR) is now composed of an Office of the Chief, the Virology and Rickettsiology Section, and the Cell Biology Section. The organizational components of LVR have remained unchanged.

The Virology and Rickettsiology Section is responsible for conducting basic and applied research concerning the prevention and treatment of viral and rickettsial infections in man, with specific emphasis on problems as they relate to the mission of DBS, such as problems concerned with vaccine safety, potency, efficacy and standardization. This Section is composed of the following 7 units: Immunoserology, Avian Leukosis, Immunofluorescence, Respirovirus, Arbovirus, Rickettsial, and Viral Pathogenesis. Nicola M. Tauraso, M.D., is Chief of this Section.

The Cell Biology Section is responsible for maintaining a facility to provide cell cultures to DBS investigators and for conducting an ongoing research program concerned with problems which might arise from the use of cell cultures for preparing virus and rickettsial vaccines. This Section is composed of the Cell Production and Cell Research Units. Henry C. Orr, Ph.D., is Chief of this Section.

B. RESEARCH ACTIVITIES

Influenza Vaccine. A major portion of the Laboratory is devoted to the execution of the Division's responsibility with regard to influenza vaccine. A constant effort is required (1) to maintain a competent Respirovirus Unit for the study of influenza viruses and other viral agents etiologically associated with respiratory tract infections of man; (2) to maintain a reference collection of infectious viral antigens and antibody reagents of influenza and other respiroviruses including those of potential importance as vaccine constituents; (3) to review and analyze manufacturers' protocols and to perform potency tests on current influenza vaccines; and (4) to improve serologic techniques for immunogenic potency testing of influenza vaccines.

We developed a quantitative radial immunodiffusion test to detect soluble residual host protein in chicken embryo-derived vaccines. Smallpox, yellow fever, and 1967 and 1968 influenza virus vaccines produced by the Sharples process had significant quantities of residual soluble host protein; 1969 influenza virus vaccines, produced by processes designed to purify the viral antigen, either had no detectable residual protein or borderline (1 mcg/ml) quantities. This immunodiffusion test will be employed on all influenza vaccine lots submitted for release in the hope that we will make progress in eliminating residual host protein in inactivated influenza vaccines.

Results of serological testing of nonhuman primate sera obtained over a four year period showed a high incidence of antibodies to influenza virus strains of the H2 and H3 hemagglutinin subtypes. This would indicate that outbreaks of type A influenza virus infection occurred in certain primate species and suggest another possible reservoir for influenza virus in nature.

Volunteer studies were performed to make comparative studies on monovalent influenza vaccines produced by different manufacturers and administered by alternative routes. The incidence of reactions was lowest among volunteers receiving the more highly purified vaccines, such as zonal purified, ether-extracted hemagglutinin and filter-purified Sharples vaccines. Interestingly, however, there was no apparent relationship between reactogenicity and measureable avian antigen and protein nitrogen.

Analysis of laboratory data showed that certain tests (i.e., CCA and neuraminidase) were quite reproducible; whereas, considerable differences were observed in the results of measuring virus particle count and protein nitrogen. Our results, however, did show that decreased protein nitrogen was associated with increased vaccine purity. Additionally, residual host protein was found to be reduced but not eliminated in these newer vaccine preparations.

More highly purified influenza virus vaccines, whether consisting of intact virions or ether-degraded hemagglutinin, induced antibody responses approximately equivalent to the cruder more reactogenic Sharples-type vaccine preparations. The presence of aluminum phosphate adjuvant, while increasing reactogenicity, did not improve the antibody response. Comparable serologic responses were found for chicken embryo chorioallantoic-derived vaccines and for vaccines prepared in bovine kidney cell cultures. Intramuscularly administered vaccine was found to be as effective as vaccine administered subcutaneously and caused less local reactogenicity. The demonstration of an inverse relationship between the pre-immunization antibody titer and the serologic response to vaccine would appear to justify the continued immunization of non-immune individuals.

Surprising as it may seem, even after influenza vaccine has been in continuous use for almost 25 years, its efficacy is much in doubt. It is our feeling that this doubt has arisen from the confusion of a multitude of contradictory reports in the literature. A critical review of this literature has revealed that most investigators have not appreciated the fact that until most recently we lacked reliable methods for quantitating the antigen content of vaccines. We strongly feel that clinical evaluation studies must be supported by a critical laboratory analysis of antigen content, and a lack of knowledge of the latter precludes any meaningful conclusion of efficacy studies in man. Our influenza vaccine program was initiated with the development of a reliable and reproducible method of quantitating vaccine antigen. With this solid foundation we are progressing to volunteer studies. We feel that our methodical approach will allow us to make some definitive conclusions on influenza vaccine efficacy.

Yellow Fever Vaccine. The DBS yellow fever project was established in April 1966 as a coordinated research program involving several laboratories in the Division. Scientific profits have been realized from this team approach to problem solving.

Studies of the antigenicity and safety of the avian leukosis virus -- (ALV) --free yellow fever (YF) vaccine primary seed have been completed. The safety and antigenicity of the ALV-free YF virus strain in monkeys and man have been established. A recommendation has been made to adopt the use of the new ALV-free YF primary vaccine seed.

Progress has been made towards the development of a cell culture derived YF vaccine. Adequate virus titers were achieved in virus-infected primary chicken embryo cell cultures. Neurovirulence studies on several cell culture-adapted virus strains are currently under way. Plans include antigenicity studies in monkeys and a clinical evaluation in man.

The finding of a suitable yellow fever virus stabilizer will enable us to lyophilize cell culture-derived yellow fever vaccine, package it in rubber-sealed ampoules, and store the vaccine at convenient temperatures.

Studies were performed to determine the effect of graded doses of 17D vaccine upon viremia, antibody response and efficacy in rhesus monkeys. There was a direct correlation between dose of inoculum and the level of antibody as measured by the HI and plaque neutralization (PN) tests. Optimal antibody responses were obtained with a vaccine dose as small as 3.4 Dex (decimal exponent) weanling mouse intracerebral LD_{50}. The significance of the PN antibody titer was extended by results of direct challenge studies. Both PN antibody titers and conversion rates were positively correlated with immunity. A neutralization index of at least 1.0 Dex reflected immunity to challenge with "wild type" virus. The HI and CF antibody titers and antibody conversion rates were not meaningfully related to vaccine dosages or immunity.

Egg Vaccines. The developing chicken embryo remains one of the most versatile substrates for vaccine manufacture. In order to maintain vaccine purity, it has been necessary to survey avian flocks for potentially troublesome microbial infections and to maintain chicken flocks which are free of known viral, bacterial and mycoplasma contaminants. The Avian Leukosis and Immunofluorescence Units of this Laboratory provide the DBS with the competency to deal with potential problems in this area.

Recently, we have been able to use the fluorescent antibody (FAB) test to detect the virus causing Marek's disease, a contagious neuroproliferative disorder which is prevalent in chicken flocks in this country. From what is already known about Marek's virus, it is highly unlikely that this virus could survive the commonly employed procedures for preparing live virus vaccines. We will soon be able to examine finished vaccine and remove the speculation concerning the potential contamination of egg derived vaccines by Marek's virus.

The Avian Leukosis Unit of this Laboratory has been responsible for monitoring the NIH ALV-free chicken flock to insure that the flock remain free from these viruses. From our collaborative studies with a USDA geneticist we recently discovered that the NIH flock was not homogeneous genetically and that, by and large, the flock was resistant to avian leukosis subgroups B viruses (i.e., C/B). We have been able to identify and segregate some chickens with respect to their genetic susceptibility. A system of cross-breeding has been established and the embryos of trap-nested eggs are currently being screened to determine their genetic status. It is our hope that, eventually, we will be able to establish a flock which will be susceptible to viruses of all subgroups (i.e., a C/0 flock). This will enable us to perform more easily our control responsibility to insure

that vaccines produced in chicken embryo derived tissues are free from contaminating avian leukosis viruses. The maintenance of an avian leukosis virus genetically susceptible flock is a time-consuming, lack-lustre chore which is not fully realized by egg users, but which is nevertheless singularly necessary for certification of a "gold-bar" flock.

Rickettsial Vaccines. As with most killed antigens, it is difficult to measure the potency of rickettsial vaccines. Emphasis was directed towards developing a candidate lot of inactivated Typhus Vaccine for use as a reference antigen. Lots of commercially produced Typhus vaccine believed suitable for a reference vaccine have been obtained and tested by the Laboratory of Control Activities (LCA), DBS and by the manufacturer. The four candidate lots of vaccine have passed the safety, purity and potency tests ordinarily required for licensing of Typhus Vaccine. Clinical trials have been initiated to evaluate the reactogenicity and antigenicity of these preparations in volunteers.

Arbovirus Studies. With abolishment of NIAID's Laboratory of Tropical Virology, the Arbovirus Unit of our Laboratory became the only facility on the NIH reservation with the professional and technical competency capable of performing studies on arboviruses. The working collection of viral and antibody reagents has expanded considerably. Present reagents representing a number of virus strains of most of the major and minor arbovirus groups are available and being utilized for routine and special studies.

Hemorrhagic Fever Studies. Previous reports described this Laboratory's isolation of the heretofore unknown virus of simian hemorrhagic fever which was responsible for a devastating epizootic of hemorrhagic fever among colonies of rhesus monkeys at the Primate Quarantine Unit, LAB, DRS (Fall, 1964), and at the Sukhumi Institute of Experimental Pathology and Therapy, USSR (July, 1964).

Our studies have revealed that the isolation of SHF virus strains in cell culture is dependent upon the use of a susceptible MA-104 cell strain and that the ability of such strains to support the replication of these viral agents may vary. Using this information we were able to isolate a viral agent in cell culture from materials derived from the Sussex/69 epizootic.

Viral Pathogenesis Studies. A new class of unusual agents found to cause diseases in man (kuru, subacute sclerosing panencephalitis) and animals (scrapie, visna, maedi) show unusual properties, such as long latent incubation period of months to years, protracted disease course, and pathologic changes unlike those of acute viral diseases. Studies with this new class of infectious agents and possible contaminants of biological products will provide DBS with the competency to develop testing and monitoring techniques.

Monkeys infected with the neuropathic measles virus developed a subclinical progressive encephalitis. Immunization preceeding the infection with the neuropathic virus prevented encephalitis. However, once

encephalitis was initiated in non-immune animals, attempts to stimulate cellular immunity with antigen incorporated into complete Freund adjuvant were of limited beneficial effect. In partially immunosuppressed animals, the disease took a lethal course 2-3 weeks after infection. Stimulation of the immune response in those animals prevented the lethal outcome but did not eliminate the virus from the CNS. Partly immunosuppressed monkeys, superinfected with pantropic measles virus in the early stage of measles encephalitis, developed a hyperacute lethal encephalitis.

Additional studies were performed to evaluate the circumstances under which measles virus displays neurotropic properties. It was found that on initial passaging of the hamster neurotropic measles virus strain in cell culture, there ensued a slowly progressing infection characterized by cell-to-cell spread and production of large syncytia. No infectious virus was released into the medium and the infected cells lacked specific determinants for hemadsorption on their surface virus. Plaque purified giant cell variants displayed different degrees of neurotropism indicating that the capability of the virus to proliferate in nervous tissue and giant cell CPE are independent properties of the virus. However, the association of these two properties combined with a defective replicative cycle appeared to be prerequisite for high neurovirulence as evidenced by encephalitogenicity in monkeys.

Knowledge of the biological properties of neurotropic strains as well as the character of the host reaction are important for a better understanding of potential preventive or curative measures.

Cell Culture Studies. A program of research and development in connection with production of cell cultures for the Division is actively pursued. Studies involved: (1) developing new and improved techniques and methods for large-scale production of cell cultures; (2) developing additional cell culture systems as potential sybstrates for vaccine purposes; (3) studying selected biochemical parameters of cells in culture as may be related to viral susceptibility; and (4) studying factors which may influence somatic variation and modulation in cell culture systems.

As a result of some of these studies, many practical improvements have been introduced into routine cultivation of cells. Increasing emphasis is being given to studying the general biology and immunobiology of cultured cells.

C. SERVICE AND CONTROL ACTIVITIES

Cell Cultures. The Cell Biology Section continues to spend most of its effort on the purely service function of providing the entire Division with quality cell cultures for research and control uses. Much effort has been made to improve the Section's service functions and to insure adequate cell production. This service results in a considerable saving to the Division and ultimately the taxpayer. All of the DBS continuous cell lines have been replaced with certified, Mycoplasma-free cells and the cell repository is operating at an efficiency which should allow almost immediate return to full scale production in the event of accident or contamination.

Production records of primary and serial cell cultures are summarized in Tables 1 and 2. In addition, 3,386 liters of media were prepared for use in the Cell Production Unit of the Section.

Maintenance of the Cell Biology Section presents a significant monetary saving to our Division. During the 1971 calendar year, if we were to purchase the materials produced by the Cell Biology Section, it would have cost approximately the following: for primary cell cultures (Table 1), $133,049.00; for continuous cell lines, $484,765.00; for a grand total of $617,714.00. Maintenance of the Cell Biology Section during this same period cost the following: for salaries, $97,000.00; for materials, $28,000.00; for a grand total of $125,000.00. The minor cost of some ancillary items and services (such as bottles and bottle washing), which would add a bit to the over-all cost of producing cell cultures, has not been included in this latter total figure. Generally, by maintaining the Cell Biology Section the Division was able to realize an over-all saving of approximately $392,714.00. The fact that the quality of cells provided by the Cell Biology Section is consistently far superior to what we have been able to obtain commercially adds to the value of the Section not readily appreciated by looking at the dollar savings only.

Avian Leukosis Testing. The Avian Leukosis and Immunofluorescence Units are responsible for testing vaccines for potential contamination with viruses of the avian leukosis complex. In addition they routinely check egg-derived vaccines for the presence of other avian contaminants (e.g., reticuloendotheliosis (RE) virus).

During this reporting period a total of 204 RIF (resistance inducing factor), FAB (fluorescent antibody), and COFAL tests were performed. Of these, 38 were tests on 16 live measles vaccines, 4 were tests on 2 epidemic typhus vaccines, 4 were tests on 1 rubella vaccine, 6 were tests on 4 Rocky Mountain spotted fever vaccines, and 7 were tests on 5 live influenza vaccines. All were found to be RIF, FAB and/or COFAL tests negative.

Our Laboratory is also responsible for monitoring the NIH RIF-free chicken flock. Numerous tests were performed on eggs from SPAFAS and the NIH flocks, egg yolks, chick embryo cell cultures, and tests to evaluate drugs for antiviral activity.

Influenza Vaccines. Table 3 summarizes the potency tests performed on influenza vaccine lots submitted during this past year. In addition, in collaboration with the Division's Laboratory of Control Activities, one reference vaccine (Lot 72) containing 700 CCA units of A/Aichi/2/68 (H3N2) and 300 CCA units B/Mass/1/71 antigens, was prepared, tested and distributed to manufacturers.

CCA Workshop. The DBS sponsored a CCA Workshop on April 17 and 18. Representatives from the active influenza virus vaccine manufacturers and from the DBS Laboratory of Virology and Rickettsiology participated in a review of the current techniques of performing the CCA test. Each participating laboratory brought his own Klett-Sommerson colorimeter; this afforded a unique opportunity to standardize each instrument against the DBS standard colorimeter. The participants expressed both their feeling that the workshop was informative and their desire to have similar meetings more frequently.

D. PUBLICATIONS

During this fiscal year, 25 articles were published from our Laboratory. Table 4 lists the senior professional staff of LVR and the numbers of articles which they have either authored or co-authored.

Table 1

Report for Calendar Year 1971

NUMBER OF PRIMARY CELL CULTURES ISSUED

Type of Culture	Suspension	Tubes	Bottles			
			2 oz.	MDB*	32 oz.	Roller**
Kidney						
Monkey, Rhesus	600	870	3,828	83	372	12
Monkey, Vervet	8,600	39,402	1,088	355	379	74
Monkey, Owl	------	------	-----	---	30	--
Rabbit	25,800	38,425	148	327	61	39
Embryo						
Mouse	------	8,068	11,690	---	---	--
Hamster	------	350	50	---	---	--
Chick	------	298	338	---	26	--

MDB* = Milk dilution bottle (8 oz.)

**Each roller bottle is the equivalent of 6 x 32-oz bottles

Table 2
Report for Calendar Year 1971

Type of Culture	NUMBER OF SERIAL CELL CULTURES PREPARED FOR ISSUE						STOCK BOTTLES MAINTAINED	
			Bottles					
	Suspension	Tubes	2 oz.	MDB	32 oz.	Roller*	32 oz.	Roller
Human								
WI-38	1,640	6,984	3,820	23	41	91	20	327
HEp-2	150	995	------	---	296	35	52	64
HeLa-S-3	90	1,971	------	14	44	10	33	20
Animal								
RK-13	------	3,950	------	---	---	--	41	327
MA-104 (C-1)	------	12,200	950	---	---	--	8	75
MA-104 (C-15)	------	12,200	6,855	---	---	--	8	157
MA-104 (C-66)	19,000	39,828	39,095	---	90	--	---	962
Vero	10,700	24,855	3,520	5	39	--	---	175
L-929	------	3,876	950	970	60	--	44	71
BHK-21	70	------	------	---	---	--	632*	93
BS-C-1	1,000	2,940	140	71	---	12	6	145
FEhL-2	------	250	210	---	---	--	72	27
Vervet Liver	850	820	------	90	---	--	760	96
Chimp Liver	600	400	------	24	---	--	760	96
Rattlesnake tumor	50	------	5	---	---	--	760	96
King snake kidney	------	150	214	---	---	--	760	96

* 422 Bottles used for media Quality Control

Table 3

Report for Calendar Year 1971
SUMMARY OF POTENCY TESTS ON INFLUENZA VACCINES

Manufacturer No.	Vaccine Type[a]	Total Lots Submitted (No.)	Lots Tested (No.)	Lots Released (No.)	Lots Rejected or Withdrawn by Mfgr.
1	CB	6	6	5	1
	Mil.	4	4	3	1
2	CB	7	7	3	4
3	CB	19	19	18	1
4	CB	7	7	6	1
5	CB	19	15[b]	19	0
6	CB	15	15	14	1
Total	CB	73	69	65	8
	Mil.	4	4	3	1

[a]CB = Civilian Bivalent; Mil. = Military formula

[b]Four lots submitted were composite, $AlPO_4$ treated samples and could not be CCA tested. Parent lots had previously passed DBS testing.

Table 4. Senior Professional Staff and Summary of Publications Realized During Fiscal Year 1971-72

Investigator	Number of Publications Senior Authorship	Co-authorship
Paul Albrecht, M.D.	2	5
Calvin G. Aulisio, Ph.D.	1	1
Mitchell Austin, M.D.	-	-
William G. Jahnes	-	1
James E. Hannah, M.D.	-	1
Richard A. Mason, D.V.M., Ph.D.	1	-
Thomas C. O'Brien, Ph.D.	6	2
Henry C. Orr, Ph.D.	1	1
Alan V. Richman, M.D.[a]	3	1
Heinz Schumacher, M.D.[b]	3	2
Nicola M. Tauraso, M.D.	7	15
Chen P. Li, M.D.	1	-

a PHS appointment expired 30 June 1971

b International Postdoctoral Fellow, appointment expired 23 June 1970

Serial No. 57
1. Virology & Rickettsiology
2. Office of the Chief
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Office of the Chief

Previous Serial Number: Same

Principal Investigator: Nicola M. Tauraso, M.D.

Other Investigators: None

Cooperating Units: None

Man Years:
Total: 4.01
Professional: 0.71
Other: 3.30

Project Description:

Objectives: Administration, planning and coordination of research and control activities of the Laboratory's sections.

The project furnishes supervisory and administrative services to all research projects and biologics control activities in the laboratory as follows: (1) over-all planning of intramural research, collaborative (contract) and control functions and their coordination among and within the several Sections of the Laboratory; (2) supervision over personnel, maintenance, travel and correspondence; (3) supervision over requisitioning and maintenance of equipment and supplies; (4) reviewing, editing and revising scientific and technical reports and manuscripts; (5) preparation of administrative reports, exhibits and other publication materials; (6) participating with other Laboratory Chiefs in staff functions for Office of the Director, including staff committees which ensure smooth operation of the Division in its many types of activity; (7) official liaison and communications with the manufacturers of viral vaccines and other biologics; (8) consultation services to individuals, academic institutions and government agencies, including liaison activities with other branches of the service, foreign governments and international agencies; (9) maintenance of reference library and reprint files.

Honors and Awards:

1. Invited participant, National Institute of Allergy and Infectious Diseases' Subcommittee on Influenza Workshop on "Influenza Virus Polypeptides and Antigens," University of Wisconsin, Madison, Wisconsin, September 20-21, 1971.

2. Invited participant, National Institute of Allergy and Infectious Diseases' Subcommittee on Influenza Workshop on "Immunologic Methodology in Influenza Diagnosis and Research," Center for Disease Control, Atlanta, Georgia, 6-7 December 1971.

3. Invited participant, National Institute of Allergy and Infectious Diseases' Subcommittee on Influenza Workshop on "Specific Immunity in Influenza," University of Washington, Seattle, Washington, 22-23 May 1972.

4. Associate Member, Commission on Influenza, Armed Forces Epidemiological Board, 6/69-6/71-6/73.

5. Member, Arbovirus Ad Hoc Committee, Research Resources Branch, National Institute of Allergy and Infectious Diseases, NIH, 1970- .

6. Fellow, Royal Society of Health, 1971- .

7. Diplomate Member in the Section on Pediatrics of the Pan American Medical Association, 1972- .

Publications:

1. Tauraso, N. M.: Review of recent eipzootics in subhuman primate colonies and their relation to man. Lab. Animal Science. In Press.

2. Tauraso, N. M.: Use of nonhuman primates for vaccine manufacturer and control. Lab. Animal Science, 21: 1072-1073, 1971.

Serial No. 139
1. Virology & Rickettsiology
2. Virology & Rickettsiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Significance of cell-mediated immune reactions in the prevention and treatment of viral infections.

Previous Serial No. Same.

Principal Investigator: Paul Albrecht, M.D.

Other Investigators: Mitchell C. Austin, M.D.
Nicola M. Tauraso, M.D.

Cooperating Units: None.

Man Years:
Total: 2.28
Professional: 1.53
Other: 0.75

Project Description:

Objectives: The sequential development and degree of cell mediated immunity in viral infections is little known and its significance, whether beneficial or detrimental, is poorly understood. The main obstacle in determining cellular immune reactions is the lack of simple and workable in vitro techniques and model infections in which these questions can be studied. The objectives are A) to modify existing methods used in cell-mediated immunity, and B) to apply them in model infections in which humoral or cellular immunity or both are experimentally altered.

Methods Employed: A. Production of immune deviation by 1) treatment with cyclophosphamid, 2) adjuvant-incorporated antigen or antigen-antibody complexes, 3) in vitro macrophage digested antigen. B. Assessing cell-mediated immunity by 1) skin test, 2) migration-inhibition factor, 3) target-cell lymphotoxicity reaction, and 4) enhancement of cell-mediated reactions by specific humoral factors.

Major Findings: We examined the suppressive and cytolytic effects of antibody and lymphoid cells on cells infected with four strains of measles virus. Two of the viruses were neuropathic for hamsters and nonhuman primates and two were pantropic strains. At different stages of infection, cell cultures were treated with reconvalescent or hyperimmune sera, non-immune or immune lymphocyte-macrophage preparations or a combination of humoral and cellular components. Spleen cells from hypersensitized animals

were virtually incapable of destroying cells infected with any of the 4 viruses. Specific antibodies suppressed the spread of virus infection. Giant cell variants (neuropathic strains) were suppressed more effectively than strand forming variants. Rather high antibody concentrations were necessary to display this effect. Combination of suboptimal antibody concentrations with spleen cells from immunized and, to a lesser extent, from unimmunized animals induced cytolysis of measles-infected cells.

Significance to Bio-Medical Research and the Program of DBS: An apprisal of the effect of immunotherapy depends, among other things, on the availability of relevant in vitro monitoring techniques. Procedures used so far were cumbersome and of limited usefulness. Modifications introducted in the reported studies and their evaluation in relevant model infections is of pressing importance at this time.

Proposed Course of the Project: These studies will continue. Our results described above will be applied in the interpretation of the pathogenesis of chronic carrier states in chronic infections of the CNS and related conditions.

Honors and Awards: None.

Publications:

1. Albrecht, P., and Schumacher, H. P.: Markers for measles virus. I. Physical properties. Arch. ges. Virusforsch. 36: 23-35, 1972.

2. Schumacher, H. P., Albrecht, P., and Tauraso, N. M.: Markers for measles virus. II. Tissue culture properties. Arch. ges. Virusforsch. In press.

Serial No. 173
1. Virology & Rickettsiology
2. Virology & Rickettsiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Pathogenesis of measles virus encephalitis (New Title)

Previous Serial Number: Same

Principal Investigators: Paul Albrecht, M.D.
Thomas C. O'Brien, Ph.D.

Other Investigators: Nicola M. Tauraso, M.D.
Alan L. Shabo, M.D.

Cooperating Units: Laboratory of Pathology, DBS

Man Years:
Total: 2.44
Professional: 1.36
Other: 1.08

Project Description:

Objectives: Measles virus has been implicated in chronic forms of encephalitis and encephalopathy in man. Measles virus with restricted growth properties was transferred into cell culture and rendered cell-free. A considerable proportion of patients with chronic measles encephalitis (subacute sclerosing panencephalitis - SSPE) has been involved in the mass measles vaccination program. However, a causal relationship of measles vaccination to SSPE has not been established. The objectives of this project are: (1) to study the biological properties of pantropic, attenuated and CNS-derived measles virus strains, and (2) to establish relevant model infections to study the immunology and immunopathology of acute and chronic measles infection.

Methods Employed: Rhesus monkeys were infected with an encephalitogenic measles virus. Groups of animals were treated with cyclophosphamide, hypersensitized with antigen incorporated into adjuvants, or superinfected with pantropic measles virus. Skin tests for delayed hypersensitivity, virus isolations, antibody analyses, histology and immunofluorescence techniques were employed. The measles virus strain neurotropic for rhesus monkeys was transferred into tissue cultures and studied during its adaptation through 26 passages to determine its reproductive cycle characteristics, neurovirulence for hamsters, plaque size and thermostability.

Major Findings: 1. Monkeys infected with the neuropathic measles virus developed a subclinical progressive encephalitis. Immunization preceeding the infection with the neuropathic virus prevented encephalitis. However, once encephalitis was initiated in non-immune animals, attempts to stimulate cellular immunity with antigen incorporated into complete Freund adjuvant were of limited beneficial effect. In partially immunosuppressed animals, the disease took a lethal course 2-3 weeks after infection. Stimulation of the immune response in those animals prevented the lethal outcome but did not eliminate the virus from the CNS. Partly immunosuppressed monkeys, superinfected with pantropic measles virus in the early stage of measles encephalitis, developed a hyperacute lethal encephalitis.

2. Previous investigations with several strains of measles have indicated that neurotropism was associated with several properties (giant cell cytopathology, larger plaque size, decreased thermostability). These properties may change on cell culture passage. To evaluate circumstances under which measles virus displays neurotropic properties, a comparative study was initiated to characterize physical and tissue culture properties of the HNT strain of measles virus at various passage levels in cell culture, both prior to and after plaque purification, and with material passaged at low and high dilutions and to determine the pathogenicity of the different cell culture passage and plaque purified materials for hamsters on intracerebral inoculation.

On initial passaging in cell cultures, the HNT strain of measles virus produced a slowly progressing infection characterized by cell-to-cell spread and production of large syncytia. No infectious virus was released into the medium and the infected cells lacked specific determinants for hemadsorption on their surface virus. Plaque purified giant cell variants of the HNT strain displayed different degrees of neurotropism indicating that the capability of the virus to proliferate in nervous tissue and giant cell CPE are independent properties of the virus. However, the association of these two properties combined with a defective replicative cycle appeared to be a prerequisite for high neurovirulence as evidenced by encephalitogenicity in monkeys.

Significance to Bio-Medical Research and Program of DBS: The increased interest in CNS infections caused by measles virus makes a search for markers associated with pathogenic properties of the virus desirable. The present results contribute to our knowledge in that they (1) determine the biological properties of a neuropathic virus able to infect and persist in the primate central nervous system, and (2) define the immunological situation before and during the encephalitic phase of infection in a primate host and the effect of an enhanced or suppressed immune response on the disease.

Proposed Course of the Project: Studies will continue at the present level.

Honors and Awards:

1. Invited participant (Dr. P. Albrecht) to the Conference on Approaches to Treatment of SSPE held Bethesda, Md. on May 21, 1971.

Publications:

1. Albrecht, P. and Schumacher, H. P.: Neurotropic properties of measles virus in hamsters and mice. J. Inf. Dis. 124: 86-93, 1971.

2. Schumacher, H. P., Albrecht, P., Clark, R. G., Kirschstein, R. L. and Tauraso, N. M.: Intracerebral inoculation of rhesus monkeys with a strain of measles virus isolated from a case of subacute sclerosing panencephalitis. Inf. Immun. 4: 419-424, 1971.

3. Schumacher, H. P., Albrecht, P., and Tauraso, N. M.: The effect of altered immune reactivity on experimental measles encephalitis in rats. Archiv. Ges. Virusforsch. In press.

Serial No. 75
1. Virology & Rickettsiology
2. Virology & Rickettsiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Studies on arthropod-borne viruses (arboviruses)

Previous Serial Number: Same

Principal Investigator: Nicola M. Tauraso, M.D.

Other Investigators: Thomas C. O'Brien, Ph.D.
Roy W. Trimmer

Cooperating Units: None

Man Years:
Total: 0.60
Professional: 0.10
Other: 0.50

Project Description:

Objectives: (1) To maintain a laboratory facility with competency in the techniques of animal work, cell culture and serology for the study of arboviruses; (2) to maintain a reference collection of arboviruses, including those of importance as vaccine candidates; and (3) to conduct studies on select arboviruses, especially those of importance as potential vaccine candidates.

Major Findings: The arbovirus facility now has the professional and technical competency capable of performing studies on the arboviruses, utilizing the techniques of animal inoculation, cell culture and serology.

The working collection of viral and antibody reagents has expanded considerably. Present reagents representing a number of virus strains for most of the major and minor arbovirus groups are available and are being utilized for routine and special studies. During this year studies were continued with emphasis on simian hemorrhagic fever, American hemorrhagic fever and yellow fever vaccine viruses (reported separately under project Nos. 118 and 121 respectively).

The effects of various commonly used procedures for removing non-specific viral inhibitors upon serum proteins and immunoglobulins were

evaluated. Several types of effects were observed. Treatment with ether, chicken red blood cells, receptor destroying enzyme (RDE), and RDE plus heat (65°C/30 minutes) resulted in a generalized increase in the immunoglobulin concentration. Conversely, treatment with RDE plus mercaptoethanol and with periodate produced a generalized decrease in immunoglobulin concentration.

Significance to Bio-Medical Research and the Program of DBS: Many of the continental states, as well as Hawaii, Alaska and Puerto Rico, possess the ecological prerequisites of potential vectors and reservoirs necessary for initiation of arbovirus epidemics. Vaccines against arboviruses of importance to the world--the U. S. personnel and travelers abroad--are being developed. Some may soon come within the purview of the Division of Biologics Standards. Further knowledge of the biochemical and biophysical properties is needed in order to appreciate more the unique features of arboviruses and before these viruses can be handled as biologic products.

Proposed Course of the Project: Research on selected arboviruses will continue.

Honors and Awards: None

Publications:

1. Tauraso, N. M., Pedreira, F. A., Spector, S. L., and Bernier, G. M.: Effect of various methods for removing nonspecific inhibitors to virus hemagglutination upon serum proteins and immunoglobulins. Archiv. ges. Virusforsch., 34:214-222, 1971.

Serial No. 118
1. Virology & Rickettsiology
2. Virology & Rickettsiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Studies on hemorrhagic fever viruses

Previous Serial Number: Same

Principal Investigator: Nicola M. Tauraso, M.D.

Other Investigators: Paul Albrecht, M.D.
Calvin G. Aulisio, Ph.D.
Roy W. Trimmer

Cooperating Units: None

Man Years:
Total: 0.40
Professional: 0.23
Other: 0.17

Project Description:

Objectives: (1) To maintain a facility for working with the important viruses causing hemorrhagic fever in the western hemisphere with emphasis on the immunological vaccine aspects; (2) to maintain practical cell culture and animal host systems and serological tools for working with this group of viruses; (3) to study the immunological relationships of these viruses using in vitro serological tests and in vivo animal protection tests; and (4) to study the pathophysiology of hemorrhagic fever using monkeys infected with simian hemorrhagic fever (SHF) virus as a model.

Methods Employed: Standard virologic and serologic methods were employed in these studies.

Major Findings: At least 6 epizootics of SHF have occurred at 4 different primate centers. Although these diseases could easily be transmitted to other monkeys of the Macaca species, difficulty has been encountered in isolating the causative virus in cell culture.

The results of our studies have shown that the isolation of SHF virus strains in cell culture is dependent upon the use of a susceptible MA-104 cell strain and that the ability of such strains to support the replication of these viral agents may vary. Using this information we were able to

isolate a viral agent in cell culture from materials derived from the Sussex/69 epizootic.

The results of our pathophysiology studies are too preliminary to report at this time.

Significance to Bio-Medical Research and the Program of DBS:
A. Concerning the studies on the American hemorrhagic fever viruses, there is no doubt that viral hemorrhagic fevers have become a major public health concern in the Americas. So far attempts to provide a safe and adequate vaccine against the Argentinian disease have been unsuccessful. During the most recent epidemics of Bolivian hemorrhagic fever, much consideration was given to the possibility of immunization as an eventual control measure. It would be in keeping with the mission of this Division to develop and maintain the competency able to cope with the problems of evaluating the potential vaccine candidates.

B. Since primates are needed by the Division in carrying out the required safety tests on some vaccines, it is only logical that the arbovirus facility of LVR maintain a competency able to cope with certain aspects of endogenous viral diseases of monkeys.

During the years of 1964 to 1969 there have been six known outbreaks of simian hemorrhagic fever. The significance of knowing more about potential hazardous diseases of monkeys is obvious.

Proposed Course of the Project: Studies on the American hemorrhagic fever and simian hemorrhagic fever viruses will be continued.

Honors and Awards: None

Publications:

1. Tauraso, N. M., Kalter, S. S., Ratner, J. J., and Heberling, R. J.: Simian hemorrhagic fever. In Medical Primatology 1970, E. I. Goldsmith and J. Moor-Jankowski (Ed.), S. Karger AG, Basel, Switzerland, 1970, pp. 660-670.

Serial No. 121
1. Virology & Rickettsiology
2. Virology & Rickettsiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Studies on Yellow Fever Vaccine (YFV)

Previous Serial Number: Same

Principal Investigators: Nicola M. Tauraso, M.D.
Richard A. Mason, D.V.M.

Other Investigators: William G. Jahnes
James E. Hannah, M.D.
Ruth L. Kirschstein, M.D.
Douglas E. Lorenz, Ph.D.
Freddie Mitchell, M.S.
Edward B. Seligmann, Jr., Ph.D.
Richard O. Spertzel, D.V.M., Ph.D.
Thomas C. O'Brien, Ph.D.

Cooperating Units: Laboratory of Control Activities, DBS
Laboratory of Pathology, DBS
U.S. Army Medical Research Institute of Infectious Diseases, Frederick, Md.

Man Years:
Total: 6.66
Professional: 2.58
Other: 4.08

Project Description:

Objectives: A. (1) To determine the relationship between the dose of avian leukosis virus (ALV)-free vaccine and viremia and antibody titers in monkeys; and (2) to determine the relationship between antibody titers and immunity.

B. (1) To develop a cell-culture derived vaccine; and (2) to evaluate the neurovirulence properties and immunogenicity of this vaccine in monkeys.

C. To evaluate virus stabilizers to protect vaccine against the deleterious effect of lyophilization and storage.

Methods Employed: Utilizing the broad resources available to the Arbovirus, Immunoserology, and Avian Leukosis Units, LVR, an attempt is being made to investigate the various aspects of this project by common laboratory techniques.

Major Findings: The DBS yellow fever project was established in April 1966 as a coordinated research program involving several laboratories in the Division.

A. Laboratory Investigations:

1. Plaque neutralizing (PN) antibody titers on triplicate serum samples from 221 monkeys demonstrated that optimal antibody responses (>90% conversion) were obtained with a vaccine dose as small as 3.4 Dex weanling mouse (WM) intracerebral LD_{50}. Below a threshold level, both seroconversion rates and antibody levels were related to vaccine dosage. The PN test was found to be a more sensitive and specific indicator of YF antibody following vaccination than either the hemagglutination-inhibition (HI) or the complement-fixation (CF) test. Protective quantities of vaccine were also dose related below a threshold level. In a challenge study involving 68 monkeys, better than 90% of the animals that had received a dose of at least 2.3 Dex WM intracerebral LD_{50} survived. The significance of the PN antibody titer has thus been extended by this observation which relates it to protection. Both PN antibody titers and conversion rates were correlated with immunity. A PN antibody titer of at least 1.0 Dex neutralization index was selected as the titer dividing susceptible from immune monkeys. The HI and CF antibody titers and percent conversion rates were not meaningfully related to vaccine dosages or immunity.

2. Additional studies were performed to determine the effect of graded doses of the 17D vaccine upon viremia and antibody response in monkeys. Graded doses of vaccine were inoculated subcutaneously into 4 different groups of rhesus monkeys. Each group of 5 animals was inoculated with either undiluted or one of the following serial 100-fold vaccine dilutions: 10^{-2}, 10^{-4}, 10^{-6}. The viremia responses were studied in 3 systems: weanling mice, and MA-104 and primary chicken embryo cell cultures. The serologic responses at 14, 28, 42, 56, and 84 days after inoculation were measured by the HI, CF, and PN tests. The results of these studies demonstrated the following: first, there was a direct correlation between dose of inoculum, and the level of antibody titer produced as measured by the HI and the PN tests; second, there was no correlation between the dose of vaccine and the time of appearance, the titer and the duration of viremia; and third, there was no correlation between viremia and serologic responses.

3. Studies on the development of a cell culture-derived YFV showed that adequate virus titers could be achieved in virus-infected primary chicken embryo cell cultures growing at 32°C, and harvesting at 3 days. Neurovirulence studies on several cell culture-adapted virus strains are currently under way.

4. A suitable YF virus stabilizer has been found. This will enable us to lyophilize cell culture-derived YFV, package it in rubber-sealed ampoules, and store the vaccine at convenient temperatures.

Significance to Bio-Medical Research and the Program of DBS: A re-evaluation of old vaccines using more up-to-date methods is one of the responsibilities of the Division. The benefit of developing more purified cell culture-

derived vaccines and updating methods of determining vaccine potency is obvious.

Proposed Course of the Project: These studies are being continued.

Honors and Awards: None.

Publications:

1. Mason, R. A., Tauraso, N. M., Ginn, R. K., O'Brien, T. C., and Trimmer, R. W.: Yellow Fever Vaccine. V. Antibody response in monkeys inoculated with graded doses of the 17D vaccine. Appl. Microbiol. In press.

2. Tauraso, N. M., Spector, S. L., and Trimmer, R.: Yellow fever vaccine. III. Antibody response in monkeys inoculated with a vaccine free from contaminating avian leukosis viruses. Archiv. ges. virusforsch. In press.

3. Tauraso, N. M., Coultrip, R. L., Legters, L. J., Richman, A. V., Rosenberg, D. M., Savadge, T. O., Shelokov, A., Spector, S. L., and Trimmer, R. W.: Yellow fever vaccine. IV. Reactogenicity and antibody response in volunteers inoculated with a vaccine free from contaminating avian leukosis viruses. Proc. Soc. Exptl. Biol. Med., 139: 439-446, 1972.

Serial No. 122
1. Virology & Rickettsiology
2. Virology & Rickettsiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Studies on respiratory viruses
I. Influenza virus vaccines

Previous Serial Number: Same

Principal Investigators: Nicola M. Tauraso, M.D.
Thomas C. O'Brien, Ph.D.

Other Investigator: James E. Hannah, M.D.

Cooperating Units: None

Man Years:
Total: 4.22
Professional: 1.55
Other: 2.67

Project Description:

Objectives: (1) To maintain a competent Respirovirus Unit for the study of influenza viruses and other viral agents etiologically associated with respiratory tract infections of man; (2) to maintain a reference collection of infectious viral antigens and antibody reagents of influenza viruses and other respiroviruses including those of potential importance as vaccine constituents; (3) to review and analyze manufacturers' protocols and to perform immunogenic potency tests on current influenza virus vaccines; (4) to improve serologic techniques for immunogenic potency testing of influenza virus vaccines; (5) to compare reproducibility of serologic tests for immunogenic potency of current influenza virus vaccines; and (6) to conduct immunogenic studies with influenza virus of epidemiologic importance in the United States.

Methods Employed: Standard virologic and serologic methods were employed in these studies.

Major Findings:

A. Laboratory Investigations.

1. A radial immunodiffusion technique was used to quantitate residual soluble host protein in chicken embryo-derived vaccines. Six replications each of the following concentrations of ovalbumin (1,2,3,4,5,6,7,8,9,10,15

20,30 and 35 mcg/ml) were tested by radial immunodiffusion. Regressions were computed using the area measurement of response. It was found that the 6 replications could be fitted to a straight line separately. A Bartlett's test for homogeniety of the variances of the 6 replications was barely significant at the 5% level. A covariance of the 6 replications showed no significant difference in the slopes, nor the adjusted means, so the data of the 6 replications was pooled to form a single straight line. Influenza virus vaccines from six manufacturers (1967, 1968, and 1969 formulations); measles virus vaccine, avianized smallpox virus vaccine, yellow fever virus vaccine, and dilutions of chicken embryo homogenate were analyzed for their concentration of egg protein. All vaccines tested were undiluted. Smallpox, yellow fever, and 1967 and 1968 influenza virus vaccines produced by the Sharples process had significant quantities of residual soluble host protein. 1969 influenza virus vaccines either had no detectable residual protein or boderline (1 mcg/ml) quantities.

In addition, compilation and analysis of the results from radial immunodiffusion testing of all influenza vaccines, in relation to manufacturer, type of vaccine, and manufacturing process, submitted from 1966 to 1971 is currently in progress.

2. Influenza A viruses isolated from human, swine, equine, and several avian species have been identified and classified based on a common internal ribonucleoprotein antigen. Serological relationships have been demonstrated between the external antigens of certain influenza virus strains of human, porcine, equine and avian origins. Furthermore, laboratory production of recombinant viruses from equine or avian strains and human influenza virus and, more recently "in vivo" production of new influenza A viruses after mixed inoculation of pigs and turkeys with antigenically distnct influenza A viruses has been demonstrated. These results have furthered the concept that human influenza pandemics may originate from animal or avian reservoirs or vice versa.

Little, however, is known about the infection of nonhuman primates with influenza strains of human origin or the possibility that simian species could act as reservoirs of human influenza strains. The results of serological testing (HI and CF) of nonhuman primate sera obtained over a four-year period showed a high incidence of antibodies to influenza virus strains of the H2 and H3 hemagglutinin subtypes. This would indicate that outbreaks of type A influenza virus infection occurred in certain primate species and suggest another possible reservoir for influenza virus in nature. Hemagglutination-inhibition (HI) testing of sera collected from African green monkeys captured in the Kenya-Tanzania area of East Africa demonstrated significant antibody titers to A/Hong Kong (HK)/68 ($H3N2$) virus in serum samples obtained 8 to 10 months prior to the first report of influenza-like illness in East Africa, and 3 to 5 months prior to the first report of outbreaks of Hong Kong influenza in southeastern China. The results suggest that certain species of nonhuman primates may be involved in the epidemiology of influenza due to their close association with human living areas.

3. Analysis of the results of hemagglutinin testing concerning an evaluation of the relative capacity of different influenza virus strains (human, equine, swine, avian, and recombinants) to elute from red blood cells of different animal species (human type-O, guinea pig, swine, horse, rhesus monkey, cockerel, turkey, and duck) at different temperatures (4°C, 22°C, 37°C) is in progress.

4. Studies are currently in progress concerning the evaluation of the relationship between chicken cell agglutinating (CCA) units and hemagglutinin (HA) titers of inactivated influenza virus vaccines.

B. Volunteer Studies.

1. A variety of new techniques for the production of influenza vaccine are now available. Zonal ultracentrifugation has led to the development of relatively homogeneous vaccines, antigenically. Tissue culture methods have been enlisted for vaccine production making possible the further reduction of local and systemic reactions caused by sensitivity to avian antigens incident to vaccine production in chicken eggs. However, these factors have also contributed to the current situation in which numerous variables among vaccines with respect to antigenic content and toxicity are compounded by various methods of preparation, routes of administration and multivalent composition.

In this light, it has been a matter of special interest to make comparative studies of monovalent influenza vaccines produced by different manufacturers and administered by alternative routes. Studies to evaluate differences in the methods of vaccine preparation and routes of administration were, therefore, performed at the Great Lakes (Illinois) Naval Training Center in collaboration with the Naval Medical Research Unit No. 4. In the first study, a total of twenty-two formalin inactivated monovalent A2/Aichi/2/68 influenza vaccine lots, containing approximately 400 chicken cell agglutinating (CCA) units per dose, were administered to approximately 2800 recruits. The vaccines were prepared by seven commercial firms by four different methods and were injected by either the subcutaneous or intramuscular routes, as were the five corresponding saline placebo preparations. Essentially, vaccines were prepared by one of four methods: The conventional Sharples techniques, zonal ultracentrifugation, and ether-extracted Sharples or zonal concentrates. All vaccine lots were prepared in the chicken embryo allantoic sac except for the zonal/HA vaccines which were derived from bovine kidney tissue cultures. Matched "sister" lots of Sharples and Sharples/HA vaccines were prepared with and without aluminum phosphate adjuvant. We included two or more vaccine lots from each manufacturer in order to evaluate lot to lot, as well as manufacturer to manufacturer, variation. Detailed laboratory analyses of the vaccines employed in this study were performed to help evaluate antigenicity and reactogenicity of the vaccine preparations. Each of the resultant 47 vaccine and placebo groups was administered to approximately 60 men.

Analysis of laboratory data showed that certain tests (i.e., CCA and neuraminidase) were quite reproducible; whereas, considerable differences were observed in the results of measuring virus particle count and protein nitrogen. These results, however, do show that decreased protein nitrogen is associated with increased vaccine purity. Evaluation of the "purity" in new techniques for production of inactivated influenza vaccines by radial immunodiffusion was employed to quantitate soluble residual host protein in both the chicken embryo-derived and bovine kidney-derived vaccines used in this study. Residual host protein was found to be reduced but not eliminated in these newer vaccine preparations.

Considerable variation in vaccine reactogenicity was found for the different manufacturing processes, among manufacturers employing essentially similar processes, and even among different vaccine lots prepared by a single manufacturer by the same process. Vaccines containing aluminum phosphate adjuvant were uniformly more reactogenic. The incidence of reactions was lowest among recruits receiving the more highly purified vaccines.

More highly purified influenza virus vaccines, whether consisting of intact virions or ether-degraded hemagglutinin, induced antibody responses approximately equivalent to the cruder more reactogenic Sharples-type vaccine preparations. The presence of aluminum phosphate adjuvant, while increasing reactogenicity, did not improve the antibody response. Comparable serologic responses were found for chicken embryo chorioallantoic-derived vaccines and for vaccines prepared in bovine kidney cell cultures. Intramuscularly administered vaccine was found to be as effective as vaccine administered subcutaneously and caused less local reactogenicity. The demonstration of an inverse relationship between the pre-immunization antibody titer and the serologic response to vaccine would appear to justify the continued immunization of non-immune individuals.

Additional volunteer studies were performed in Denver, Colorado. In this study, asthmatic children residing at the Children's Asthmatic Research Institute and Hospital, Denver, Colorado, were given either a 0.25 ml. or a 0.5 ml. subcutaneous injection of a bivalent influenza virus vaccine manufactured by National Drug Company containing 700 CCA units per 0.5 ml. [400 CCA units of A/Aichi/2/68 (H_3N_2) and 300 CCA units of B/Mass/3/66]. The children were observed immediately and at 8, 24, and 48 hours post injection for systemic and local reactions. No child developed a systemic reaction. Except for two children who each developed severe local reactions to a dose of 0.5 ml., most of the children had either no reaction or a mild reaction characterized by tenderness to palpation and/or swelling. In children aged 9 to 15 years there was little difference in degree of reaction between those receiving 0.25 ml. and those receiving 0.5 ml. Furthermore, children on steroids experienced no less a degree of reactiveness than those not on steroids.

Significance to Bio-Medical Research and the Program of DBS: DBS is responsible for the control and potency testing of current influenza virus vaccines. Sustained and comprehensive scientific research, as performed by the Respirovirus Unit, is essential for the improvement and development of antigenically competent influenza virus vaccines.

Proposed Course of the Project: Studies on influenza viruses and influenza vaccines will continue.

Honors and Awards:

1. Invited participant (Dr. O'Brien), National Institute of Allergy and Infectious Diseases' Subcommittee on Influenza Workshop on "Immunologic Methodology in Influenza Diagnosis and Research," Center for Disease Control, Atlanta, Georgia, 6-7 December 1971.

Publications:

1. O'Brien, T. C., Maloney, C. J., and Tauraso, N. M.: Quantitation of residual host protein in chicken embryo-derived vaccines by radial immunodiffusion. Appl. Microbiol., 21:780-782, 1971.

2. Edwards, E. A., Peckinpaugh, R. O., Rosenbaum, M. J., Mammon, R., Tochman, M., Frazier, W., Tauraso, N. M. and Myers, M. G.: Influenza vaccine: Primary and booster response to influenza vaccine by dose and route of administration. Discussion in Proceedings International Conference on the Application of Vaccines Against Viral, Rickettsial, and Bacterial Diseases of Man, Scientific Publication No. 226, Pan American Health Assoc., Washington, D. C., 1971, pp. 389-391.

3. Richman, A. V., and Tauraso, N. M.: Attempts to demonstrate hemagglutination and hemadsorption by respiratory syncytial virus. Appl. Microbiol., 21:1099-1100, 1971.

4. Richman, A. V., and Tauraso, N. M.: Growth of respiratory syncytial virus in suspension cell cultures. Appl. Microbiol. 22:1123-1125, 1971.

5. O'Brien, T. C., and Tauraso, N. M.: Antibodies to type A influenza viruses in sera from nonhuman primates. Bull. W.H.O., In press.

Serial No. 209
1. Virology & Rickettsiology
2. Virology & Rickettsiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Evaluation of the relative immunogenicity of two live virus vaccines given at various intervals

Previous Serial Number: Same

Principal Investigators: Nicola M. Tauraso, M.D.
Thomas C. O'Brien, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:
Total: 1.13
Professional: 0.21
Other: 0.92

Project Description:

Objectives: (1) To evaluate the relative immunogenicity of two live virus vaccines (i.e., Smallpox and Yellow Fever Vaccines) given at various intervals. (2) To evaluate and improve methods for serological testing of smallpox vaccines.

Methods Employed: Standard virologic and serologic methods were employed in these studies.

Major Findings:

A. Laboratory Investigations

1. Although the vaccinia plaque neutralization assay system in rhesus monkey kidney monolayer cell cultures is now well established, there is considerable confusion in the literature as to the optimum temperature-time period for vaccinia virus neutralization: 4°C - 1 hr; room temperature - 1 hr; room temperature - 24 hr; room temperature - 4 days; 37°C - 30 min; 37°C - 70 min plus 37°C - 10 min with goat anti-rabbit γ-globulin; 37°C - 2 hr; 37°C - 2 hr followed by 4°C overnight; and 37°C - 24 hr. We were confronted with this confusion when we attempted to select the ideal vaccinia virus neutralization test for use in our laboratory. Consequently, we were prompted to study the kinetics of vaccinia virus neutralization in a plaque assay system in order to determine the optimal temperature and length of incubation of the virus-antibody mixture.

Vaccinia virus plaque neutralization performed in 2 oz. bottle cultures of the MA-104 embryonic rhesus monkey kidney cell line showed that the neutralization of vaccinia virus with immune rabbit serum occurred optimally at 37°C for 16-24 hours.

2. A heat-labile serum factor which enhanced the neutralizing activity of heated immune serum had been reported by early investigators. The factor which was responsible for this phenomenon had been attributed to either complement, properdin, or a nonspecific heat-labile substance.

To determine more precisely what factor in normal serum was responsible for this enhancement of the vaccinia virus neutralizing activity of heated (56°C for 30 min) immune serum, studies were initiated to investigate the effect of various reagents and treatment procedures which specifically inactivated certain components of complement, the effect of adding normal serum to immune serum before and after heat-inactivation, the effect of this factor on the kinetics of the virus-antibody reaction, and the requirement for this factor in the HI test procedure.

The results indicated that a heat-labile accessory factor present in normal serum will replace the vaccinia virus neutralizing activity of heated immune serum. Treatment of serum samples with ammonium hydroxide or cobra snake venom did not significantly reduce the activity of immune serum. Also, inactivation studies with the properdin deficient (PR) fraction of immune serum after zymosan treatment did not alter the neutralizing activity of immune serum. Although the neutralization kinetics of vaccinia virus were altered in the presence of the accessory factor, the optimum conditions for neutralization were not changed. In the HI test procedure, the addition of normal serum did not replace the HI activity lost by heating (56°C for 30 min) vaccinia immune rabbit serum. It appeared that this heat-labile accessory factor was not complement nor properdin, was required for the complete neutralizing activity of immune serum to vaccinia virus, and was not required for HI activity.

3. Although the specificity of the vaccinia virus HI test has been confirmed by several investigators, there is some disagreement as to the optimum temperature and time of incubation of the hemagglutinin-serum mixture: room temperature - 1/2 hr; room temperature - 1 hr; 37° - 1/2 hr; 37° - 1 hr; and 37° - 2 hr. Furthermore, several host systems have been employed in the preparation of the vaccinia hemagglutinin: chorioallantoic membrane (CAM) of chicken embryos; rabbit dermis; calf dermis; sheep lymph; rabbit testis; primary rhesus monkey kidney cell culture; primary mouse embryo fibroblast culture; KB cell culture; and HeLa cell culture. Confronted with these variables when we attempted to select the ideal vaccinia virus HI system for use in our laboratory, we were prompted to study the kinetics of vaccinia virus HI test in order to determine (a) the optimal temperature and length of incubation of the hemagglutinin-serum mixture and (b) a convenient method for the preparation of the vaccinia hemagglutinin.

The optimum temperature and time of incubation of the vaccinia hemagglutination-inhibition test were 37° and 16 hr respectively. A convenient method for preparing vaccinia HA antigen consisted of infecting one-day-old primary CE fibroblast monolayer cultures with a high multiplicity of vaccinia virus, employing a maintenance medium devoid of serum, preparing a 20X concentration of virus-infected cells, and finally extracting the 20X antigen homogenate with genetron.

B. Volunteer Studies

It has been generally recommended that live virus vaccines be given at least 1 month apart whenever possible. The theoretical rationale for this has been that more frequent and severe adverse reactions as well as diminished antibody responses might result. If the 1-month interval is not feasible, as with the threat of concurrent exposures or disruption of immunization programs, the vaccines should preferably be given on the same day--at different sites for parenteral products. Avoidance of the interval of about 2 days to 2 weeks has been recommended because interference between the vaccine viruses might possibly occur then.

Field observations, however, have not tended to support these theoretical considerations. Previous studies have shown that Measles and Smallpox Vaccines can be administered simultaneously, either as a single injection of combined antigens or as independent antigens at separate sites, without affecting immunogenicity as measured by seroconversions and without the potentiation of clinical reactions beyond those observed when each vaccine was given alone. Previous workers have demonstrated that live Measles, Smallpox and Yellow Fever Vaccines could be simultaneously administered safely and effectively to children.

In order to determine whether interference occurs, a study was conducted in 2 phases. In Part I, individuals were immunized initially with Smallpox Vaccine and then administered Yellow Fever Vaccine to groups simultaneously and 3, 7, 14 and 28 days later. Sera was obtained prior to and 28 days after smallpox vaccination and 28 days after yellow fever vaccination.

Part II of the study was essentially similar except that Yellow Fever Vaccine was administered initially and the time of smallpox vaccination varied as indicated above.

The results showed that the reactogenicity and antigenicity of live smallpox and yellow fever vaccines were unaffected by the interval between inoculation.

Honors and Awards: None

Publications:

1. O'Brien, T. C., Myers, M. G., and Tauraso, N. M.: Kinetics of the vaccinia virus plaque neutralization test. Appl. Microbiol. 21: 968-970, 1971.

2. O'Brien, T. C., and Tauraso, N. M.: Vaccinia Virus: Kinetics of the hemagglutination-inhibition test and preparation of hemagglutinin. Arch. Ges. Virusforsch. 36: 158-165, 1972.

3. Tauraso, N. M., Myers, M. G., Nau, E. V., O'Brien, T. C., Spindel, S. S., and Trimmer, R. W.: Effect of interval of inoculation upon antigenicity of live smallpox and yellow fever vaccines in man. J. Inf. Dis. In press.

Serial No. 325
1. Virology & Rickettsiology
2. Virology & Rickettsiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Latent viral infections in primates

Previous Serial Number: None

Principal Investigators: Thomas C. O'Brien, Ph.D.
Paul Albrecht, M.D.

Other Investigators: Nicola M. Tauraso, M.D.
James E. Hannah, M.D.

Cooperating Units: None

Man Years:
Total: 1.34
Professional: 1.01
Other: 0.33

Project Description:

Objectives: To study latent virus infections of nonhuman primate tissues, as these relate to the use of primate tissues for vaccine substrates.

Methods Employed: In addition to co-cultivation of cells from rhesus monkey tissues with uninfected Vero cells, standard virologic and serologic methods were employed in these studies.

Major Findings: 1. Previous studies have indicated that natural foamy virus infection of rhesus monkeys is type specific (type 1), that foamy viruses have been isolated from the throat as well as the kidney of rhesus monkeys, but have not been isolated from other tissues or excreta, and that carrier cultures of foamy type 1 have been established suggesting that certain foamy virus infections are regulated whereby virus can be released from cells without causing cell death. Further, no inclusion body formation associated with foamy virus CPE has been observed. No previous investigators have performed a comparative study of tissues from several measles free rhesus monkeys to isolate and serologically identify foamy viruses and to examine histochemically foamy virus cytopathology in these tissues.

2. Continuing studies in our laboratory on the optimum conditions for dispersing and cultivating brain cells "in vitro" to study the course of measles encephalitis in rhesus monkeys and to isolate measles virus from brain tissue have resulted in the isolation and serologic identification of both simian foamy

virus types 1 and 2 in primary rhesus monkey brain cell cultures. Due to the lack of attention paid to the "in vitro" cultivation of various rhesus monkey tissues and subsequent identification of the types of foamy viruses found in these tissues, comparative studies dealing with the isolation and identification of foamy virus serotypes from primary rhesus monkey brain, spleen, and kidney cell cultures were initiated. Cross neutralization tests showed the presence of foamy virus serotypes 1 and 2 in primary rhesus monkey brain, spleen, and kidney cell cultures. The frequency of isolation of foamy viruses from brain and spleen cell cultures was 86%. In addition, histochemical examination of some primary brain cell cultures and brain cell-Vero co-cultures revealed filamentous acidophilic inclusions in the cytoplasm associated with foamy virus CPE. On passage the capacity to form these cytoplasmic filaments diminished rapidly and none could be observed after three consecutive passages.

Significance to Bio-Medical Research and Program of DBS: Although several studies have shown the isolation of foamy virus type 1 from primary rhesus monkey kidney cell cultures, this is the first report of the isolation of foamy virus type 1 from tissues other than kidney, of the isolation of foamy virus type 2 from rhesus monkeys, and of the presence of inclusion bodies associated with foamy virus CPE. These studies, as well as studies by other investigators, indicate that many viruses are commonly latent in "normal" tissues from nonhuman primates and that the internal organs of these animals cannot be considered microbiologically sterile. In addition, at present, the only exception of the presence of viron RNA-dependent DNA polymerase (reverse transcriptase) activity and oncogenic potential is the foamy virus.

Proposed Course of the Project: To more precisely define the pathogenic role of foamy viruses, if any, and their mode of transmission, the following approaches have been proposed: (1) attempt to activate foamy virus type 5 from a carrier culture state in Vero cells with 5-iododeoxyuridine and dimethyl sulfoxide; (2) attempt to cause overt infection with foamy viruses in immunosuppressed (x-irradiated, cytoxan or ALS treated) laboratory animals; and (3) cultivation of tissues from a pregnant rhesus monkey and its fetus, delivered by Caesarian, separately "in vitro" and isolation of foamy viruses from these tissues.

Honors and Awards: None.

Publications:

1. O'Brien, T. C., Albrecht, P., Schumacher, H. P., and Tauraso, N. M.: Isolation of foamy virus types 1 and 2 from primary rhesus monkey brain cultures. Proc. Soc. Exp. Biol. Med. 137: 1318-1323, 1971.

2. O'Brien, T. C., Albrecht, P., Hannah, J. E., Tauraso, N. M., Robbins, B., and Trimmer, R. W.: Foamy virus serotypes 1 and 2 in rhesus monkey tissues. Arch. ges. Virusforsch. In press.

Serial Nos. 59, 65, 82
1. Virology & Rickettsiology
2. Virology & Rickettsiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Studies on rickettsiae

Previous Serial Numbers: Same

Principal Investigator: Richard A. Mason, D.V.M., Ph.D.

Other Investigators: Robert K. Ginn, B.S.
Edward B. Seligmann, Jr., Ph.D.
Nicola M. Tauraso, M.D.
Richard P. Wenzel, M.D.

Cooperating Units: Laboratory of Control Activities, DBS
Naval Medical Field Research Laboratory
Camp Lejeune, North Carolina

Man Years:
Total: 1.5
Professional: 1.0
Other: 0.5

Project Description:

Objectives: To evaluate the antigenicity of inactivated epidemic typhus vaccine of varying potencies in man, establish the minimum potency level based on clinical data and to select one of four candidate lots of vaccine as a reference against which new lots of vaccine would be compared.

Methods Employed: Clinical evaluation is presently underway. Two lots of vaccine have been administered to volunteers whose serologic responses to vaccination are being evaluted.

Major Findings:

A. Laboratory Studies.

The antigenic contents of the four candidate lots of vaccine, as determined in repeated direct CF antigen tests, are shown in Table 1. Two lots, A and B, meet or exceed the 1:40 minimum direct CF antigen content desired in the reference vaccine. The direct CF antigen contents and the total nitrogen values, also shown in Table 1, reflect the two-fold dilutions of vaccine lots prepared from lot 2A.

Repeated potency tests of vaccine lots 2A - 2D indicated average potencies of 1:214, 1:181, 1:121 and 1:90, respectively.

B. Clinical Studies.

Serologic responses in man to two lots of vaccine are presently being tested. Results will be presented when tests are completed.

Significance to Bio-Medical Research and Program of DBS: Selection of a reference typhus vaccine is essential as another means of insuring that only potent lots of new vaccine are approved and as a standard reagent for use in rickettsial research.

Proposed Course of the Project: This project will be continued during the coming year. In addition, field trials designed to evaluate the efficacy of inactivated as well as modified live typhus vaccine are needed and proposals to this effect will be submitted.

Honors and Awards:

1. Associate member (Dr. Mason), Commission on Rickettsial Diseases, Armed Forces Epidemiological Board 6/69-6/71-6/73.

Publications: None.

Table 1. Direct CF Antigen Titers and Total Nitrogen Values of Candidate Lots of Inactivated Epidemic Typhus Reference Vaccine

Test No.	Pos. Ag Control	Vaccine Lot Number A	B	C	D
1	160/160[a]	80/80	40/40	20/40	<10/10
2	160/160	80/40	40/40	20/10	10/20
3	160/160	80/80	40/40	20/20	10/20
Ave. Titer	160	80	40	20	10
Total Nitrogen (mg/ml)		0.170	0.084	0.041	0.021

a Results of duplicate tests.

Serial No. 117
1. Virology & Rickettsiology
2. Virology & Rickettsiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Avian leukosis viruses in relation to human vaccines

Previous Serial Number: Same

Principal Investigator: William G. Jahnes

Other Investigators: Calvin G. Aulisio, Ph.D.
Lyman B. Crittenden, Ph.D.
Francis J. Judge, D.V.M.
Carl T. Hansen, D.V.M.
C. P. Li, M.D.
Amos E. Palmer, D.V.M.
Nicola M. Tauraso, M.D.

Cooperating Units: Laboratory Aids Branch, DRS
Poultry Research Branch, ARS, USDA

Man Years:
Total: 2.61
Professional: 1.19
Other: 1.42

Project Description:

Objectives: (1) To test live and inactivated vaccines prepared in the chicken embryo or from chicken or duck embryo cell cultures for the presence of avian leukosis viruses (ALV); (2) to develop new and more sensitive methods of detecting viruses of the avian leukosis complex and Marek's disease; (3) to compare the relative effectiveness of the RIF (Resistance-Inducing Factor), COFAL (complement-fixation for avian leukosis) and FAB (fluorescent-antibody) tests for the detection and assay of several members of the avian leukosis complex; (4) to assist in the development of RIF-free vaccines prepared in chicken embryos or chicken and duck embryo cell cultures; (5) to determine genetic resistance to the avian leukosis viruses and to develop at the NIH Farm and Building 14G by selective cross-breeding an ALV-free chicken flock which is homogeneously susceptible to the avian leukosis viruses; (6) to continue studies on the inhibition of viral oncogenesis through the use of clam extracts and chemical inhibitors.

Methods Employed: Standard and modified cell culture techniques, complement fixation, and FAB tests were employed. Standard animal inoculation techniques were also employed.

Major Findings: 1. The testing as outlined in Objective No. 1 has been modified to include new or improved vaccines made in RIF-free chicken eggs, namely Rocky Mountain spotted fever, live, influenza "Q" fever and epidemic typhus.

During this reporting period a total of 204 RIF tests were performed. Of these 38 were tests on 16 live measles vaccines, 4 were tests on 2 epidemic typhus vaccines, 4 were tests on 1 rubella vaccine, 6 were tests on 4 Rocky Mountain spotted fever vaccines, 7 were tests on 5 live influenza vaccines. All were found to be RIF, FAB and/or COFAL tests negative. Included in the miscellaneous category were some 45 tests on 128 chicken sera for the NIH RIF-free flocks and some 100 tests on drugs submitted for viricidal effects, stock virus titrations and cell cultures.

It should be further noted that in the process of this testing and that of other investigators some 780-32 oz bottles of primary CETC of different lots from NIH RIF-free flocks and 280-32 oz bottles from different lots of SPAFAS RIF-free eggs were prepared.

2. Concerning Objective No. 2, this work has continued with emphasis on viruses of the avian leukosis complex in screening selected primary and continuous cell cultures. Recent published work by other investigators has shown that susceptibility tests of these lines to Marek's disease has been well covered with negative results and our continuing efforts have been mainly concerned with improved detection test methods.

3. Objective No. 3 has been the comparative effort of the two investigators utilizing the DBS standard RIF test procedure, the fluorescent antibody test (FAB) and the COFAL test on a comparative basis. Attempts to prepare antibodies to several strains of RSV in two species of pigeons has shown, under conditions of the experiments, that the pigeon system is as difficult to produce suitable RSV antibodies as in the hamster system. (See Report No. 164).

4. Objective No. 4 concerned studies with the RIF-free yellow fever vaccine, (a) evaluation of storage viability and of stabilizers, (b) preparation of RIF-free yellow fever vaccine in chicken embryo cell cultures (See Report No. 121), (c) a further study was conducted to determine the comparative sensitivity of "in vivo" and "in vitro" methods of RIF virus isolation. Some 124 RIF tests were run on 172 chicken sera and another 79 tests on the vaccine itself. Preliminary incomplete results indicate that the "in vitro" method might be slightly more sensitive.

5. Objective No. 5 was to determine the extent of the genetic resistance to the different sub-group avian tumor viruses (A-B-C-D) in the NIH RIF-free flock and the possible correction through proper in-breeding of susceptible chickens (those whose embryos support the growth of subgroups A-B-C-D avian leukosis viruses).

A major portion of our screening effort has been to monitor the two NIH RIF-free flocks at the NIH Farm in Poolesville and on the original flock in Building 14G. Included in this screening was the aforementioned effort to correct the inbred genetic resistance to sub-group "B" (C/B) avian leukosis tumor viruses to that of genetic susceptibility to all sub-groups of avian leukosis viruses (C/O). This project was performed with the valuable collaboration of Dr. Lyman Crittenden, geneticist of USDA, Beltsville, Maryland and Drs. F. Judge, C. Hansen, and A. Palmer of the Division of Research Services, NIH. In the flock, 2/3 of the hens were shown to be producing resistant embryos (C/B). Consequently, a system of cross-breeding had to be established and this was accomplished by getting in-bred line 6 C/O eggs from the USDA Regional Poultry Laboratory, East Lansing, Michigan and hatching them. The resistant homozygous line 6 inbred roosters were mated with heterozygous NIH hens to produce susceptible eggs. One isolation has been established and a second one is being genetically monitored. It should be noted that due to other contingencies, the Building 14G flock is slowly being phased out and only the NIH Farm flock will remain as an in-house source of RIF-free eggs.

6. Concerning Objective No. 6, studies were conducted on the effects of paolins 1 and 2, Chinese plant extracts and other chemical inhibitors on the growth and transforming ability of Rous sarcoma virus in cell cultures.

Significance to Bio-Medical Research and the Program of DBS: At present, three tests are routinely performed on vaccines for detecting the viruses of the avian leukosis complex, these are the RIF, FAB and COFAL tests. The latter two tests are more rapid and provide a double check on vaccine testing with the FAB test providing a quick and easy method for monitoring of RIF-free flocks. Since RIF-free chick embryo fibroblasts are required for the safety testing of vaccines, a RIF-free chicken flock must be maintained at NIH. The monitoring of this flock can be rapidly and easily achieved by using techniques to test eggs for maternal antibody by the indirect FAB technique. The development of more sensitive and more reliable procedures is of utmost importance to the Division's mission. The development of satisfactory tests for the agent of Marek's disease and the importance of this disease in vaccine production is essential to the Division's purpose. The maintenance of an avian tumor virus genetically susceptible RIF-free flock is of great importance for testing and for production of experimental vaccines.

Proposed Course of the Project: Work will continue on improving the present RIF, FAB, and COFAL tests and on developing simpler and more rapid procedures for the detection of the avian leukosis viruses. Studies on Marek's disease, an important cause of avian leukosis, will be continued in the hope of developing a satisfactory test system. Studies on selective inbreeding to correct resistance to susceptability (C/O) of the NIH flock will be continued.

Our studies on avian leukosis viruses and the potential problem of their contaminating egg-derived vaccines will be reported in the future as part of a larger over-all study on endogeneous viruses contaminating avian eggs (Report No. 164).

Honors and Awards: None

Publications:

1. Richman, Alan V., Aulisio, Calvin G., Jahnes, William G. and Tauraso, Nicola M.: Avian leukosis antibody response in individuals given chick embryo derived vaccines. Proc. Soc. Exptl. Biol. Med. 199:235-237, 1972.

Serial No. 164
1. Virology & Rickettsiology
2. Virology & Rickettsiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Studies on endogenous viruses contaminating avian eggs and other cell substrates used in vaccine manufacture.

Previous Serial Number: Same

Principal Investigator: Calvin G. Aulisio, Ph.D.

Other Investigators: William G. Jahnes
Nicola M. Tauraso, M.D.

Cooperating Units: None

Man Years:
Total: 1.17
Professional: 0.59
Other: 0.58

Project Description:

Objectives: A. To evaluate and develop improved methods for detection of endogenous agents in chicken and duck eggs employed in vaccine manufacture: (1) Induction of avian tumor viruses by employing the chicken helper factor (chf) and by using physical and chemical means; (2) evaluate the sensitivity of the standard methods (FAB, C-F) for avian tumor virus detection with the new chf test; (3) evaluate the influence of bacterial endotoxins in Marek's disease; (4) characterize and identify the fluorescing substances in duck embryo cell cultures; and (5) provide service and guidance to other laboratories monitoring avian leukosis viruses in vaccines and chicken flocks.

Methods Employed: These studies require the use of the following: standard and modified cell culture techniques; immunofluorescence, complement-fixation, and chf tests; animal inoculation, interference, histopathology, and standard bacteriological techniques.

Major Findings: 1. Hanafusa, et al., Proc. Nat. Acad. Sci. USA, 66: 314 (1970), described the existence of chf in most, if not all, chick embryo cells. In these cells both group-specific internal and viral specific-envelope antigens are regularly synthesized although mature virus appears not to be. Weiss, et al., Virology 46: 920 (1971), induced avian tumor viruses in normal chicken cells with ionizing radiations or chemical carcinogens and mutagens. The implication of these findings has importance to the Division's

mission, especially since the induced avian leukosis viruses belong to the subgroup E which is difficult to detect in both the RIF and C-F tests. Tests employing 20-methyl-cholanthrene and 5-bromodeoxyuridine with chicken and duck cells have been started in an attempt to activate latent agents. The results of these tests are still preliminary.

2. In a cooperative study by the Immunofluorescence, Avian Leukosis, and Immunoserology Units, we were able to evaluate the comparative sensitivity of FAB, RIF, and CF tests for detecting avian leukosis viruses of subgroups A and B. The immunofluorescence test detected earlier preparations containing little antigen. The RIF and CF tests later became positive as the antigenic content increased. Hanafusa demonstrated chf in more than 70% of the embryos from leukosis-free flocks. The chf could be a cryptic form of ALV remaining in cells as an integrated gene unharmful to the hosts. As we develop competency in this area (chf) an evaluation of the sensitivity of the chf test will be determined. A re-evaluation of the reported absence of RIF in YF vaccine prepared in RIF-free embryos assumes importance.

3. A suspension of chicken kidney cells containing a herpes type virus when injected into chickens produces Marek's disease. Most of the moribund and dead birds have multiple tumors in one or more of the organs. Some of the birds, although having typical Marek's clinical signs were free of tumors and other discernable lesions in any of the organs. These findings suggest other factors might play roles in the overt manifestation of Marek's disease in infected chickens.

Further, evidence that neither antibody nor interference protects chickens vaccinated with attenuated Marek's vaccine postulates another possible mechanism responsible for Marek's symptoms. The possibility exists that intestinal flora might produce bacterial endotoxins that influence clinical signs associated with Marek's disease in chickens.

4. Serum from a rabbit immunized with chicken CELO virus fluoresced the cytoplasmic granular substances in duck embryo cell cultures. Normal rabbit serum and sera from rabbits immunized with human adenovirus types 3, 4 and 7 did notfluoresce these cytoplasmic substances. This observation has renewed interest in the cytoplasmic fluorescing substances in duck cells. Attempts to identify and characterize these substances are in progress.

5. During this reporting period approximately 250 RIF test samples (from the Avian Leukosis Unit), 60 samples (Yellow Fever Project), and 200 serum samples (from the RIF-free flock, NIH) were examined by the immunofluorescence test for avian leukosis virus. In addition the Immunofluorescence Unit provided service in studies with influenza, simian hemorrhagic fever, simian foamy virus and respiratory syncytial virus.

Significance to Bio-Medical Research and the Program of DBS: Some licensed and experimental viral and rickettsial vaccines are prepared in chicken and duck eggs. Therefore, it is important and urgent that the

Division takes the thrust in employing new methods for detecting endogenous agents in avian substrates used in vaccine manufacture.

Proposed Course of Project: Studies to evaluate and develop improved methods for detection of endogenous agents in chicken and duck eggs will continue. Further work with Marek's herpesvirus will also continue.

Honors and Awards: None

Publications:

1. Richman, A. V., Aulisio, C. G., Jahnes, W. G., and Tauraso, N. M.: Avian leukosis antibody response in individuals given chicken embryo derived vaccines. Proc. Soc. Exp. Biol. and Med. 139: 235-237, 1972.

2. Aulisio, C. G., and Shelokov, A.: Attempted application of avian viral antibody test for detection of other microbial infections. Avian Diseases. (In press).

Serial No. 83
1. Virology & Rickettsiology
2. Cell Biology Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Studies on the biology of cultured cells

Previous Serial Number: Same

Principal Investigator: Henry C. Orr, Ph.D.

Other Investigators: Ruth C. Dunlap, Ph.D.
Richard A. Mason, D.V.M., Ph.D.
Peter G. Probst
Joseph L. Rogers
Nicola M. Tauraso, M.D.

Cooperating Units: Laboratory of Viral Immunology, DBS

Man Years:
Total: 1.4
Professional: 1.0
Other: 0.4

Project Description:

Objectives: A. Studies are designed to increase our knowledge of cells in culture. Some are short-term in nature and the results therefrom are immediately applicable to cell culture production problems. Other studies are pursued in more depth and are of a more fundamental nature with implications of a broader scope. Presently, these studies are grouped under the following subheadings: (1) Development of additional cell culture systems as potential substrates for vaccine purposes; (2) study of selected biochemical parameters of cells in culture as may be related to viral susceptibility; (3) study of factors which may influence somatic variation and modulation in cell culture systems.

Methods Employed: As applicable to the particular study, use is made of radioactive isotope labeling, thin layer and column chromatographic techniques, and various microscopic procedures as well as cytochemical and biochemical micromethods.

Major Findings:

1. Development of Cell Substrates.

a. Reptilian cell lines. Several cell lines derived from snakes have successfully been developed and partially characterized. One line of cells

is from a fibroma of a rattlesnake. These cells are free from indigenous viral agents as shown by electron microscopy and grow well under standard conditions found optimal for mammalian cell cultures except that snake cells grow best at 30°C. The cells have a fibroblast-like appearance and presently are diploid with the 2n complement of 36 chromosomes.

b. Vervet monkey liver cell lines. A combination of dilution and use of the micro-tissue culture plates has allowed us to isolate from a vervet liver before culture, two distinct cell types. One cell was epithelioid and the other a fibroblastic cell. These separate cell types were grown in the micro-plates from primary culture to roller bottle quantities; were frozen in liquid nitrogen and are being characterized as to some of their biologic properties. Karyological, cytochemical and microbiochemical studies are being performed to determine whether the epithelioid cell has characteristic properties of a "true" hepatocyte. In addition, collaborative studies on the sensitivity of the cell line for a number of viruses in in process, and electron microscopic studies are being made to detect glycogen and/or microbodies, typical of liver parenchymal cells.

c. Other mammalian tissues. Attempts are being made to apply the above techniques to isolate from tissues distinct cell types at the time of inoculation into cell culture. Livers from rabbits, human, chimpanzees, mice, as well as chicken embryos and monkey brain have been tried with various degrees of success.

2. Biochemical Parameters of Cells in Culture.

Turnover of phospholipids of cells in tissue culture under several conditions. Rates of incorporation of 32_{P_i} into sphingomyelin, phosphatidyl choline and phosphatidyl ethanolamine of normal human skin fibroblast cells in culture and those from a patient with a lipid storage disorder, Niemann-Pick disease, were compared. Extracted lipids from cells exposed for 24 hours to 100 µCi of 32_P-phosphoric acid in Tris-HCl-buffered cell culture medium without serum were separated and resolved by chromatographic procedures into the three major classes of the polar phospholipids. Each of these classes was assayed for radioactivity and its phosphorus content. The sphingomyelin fraction of the Niemann-Pick cells contained 50% more phosphorus than did sphingomyelin from the normal skin fibroblast cells. However, the specific activity of this component of Niemann-Pick cells was 32-35% of that found in the normal cells. There were no differences in the incorporation of 32_{P_i} into the other phospholipids of the diseased or normal cells. The fact that Niemann-Pick cells contain more sphingomyelin than do normal skin fibroblast cells but synthesize this lipid more slowly suggests a possible negative feedback mechanism in lipid metabolism of Niemann-Pick cells.

Significance to Bio-Medical Research and the Program of DBS:

Additional well-defined in vitro host systems are needed for current vaccine production and for vaccine testing. There is also a need for new and well defined cell-host systems for future fastidious agents. In addition, information gained from studies in this project provides the direct and immediate basis on which changes in methodology are made in keeping with the goals of the Section in producing mass quantities of high quality cell cultures.

Proposed Course of the Project: A study of the biology of cultured cells is a continuing project and on-going investigations are developed in the light of greater knowledge, need and feasibility.

Honors and Awards: None

Publications:

1. Orr, H. C., Harris, L. E., Jr., Bader, A. V., Kirschstein, R. L., and Probst, P. G.: Cultivation of cells from a fibroma in a rattlesnake, Crotolus horridus. J. Nat. Cancer Inst. 48: 259-264, 1972.

Serial No. 67
1. Virology & Rickettsiology
2. Cell Biology Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Standardization of techniques applicable to large-scale production of cell cultures

Previous Serial Number: Same

Principal Investigator: Henry C. Orr, Ph.D.

Other Investigators: Peter G. Probst
Joseph L. Rogers
Nicola M. Tauraso, M.D.

Cooperating Units: None

Man Years:
Total: 5.9
Professional: 1.7
Other: 4.2

Project Description:

Objectives: (1) To develop new and improved techniques and methods for large-scale production of cell cultures; (2) to prepare cell cultures for use by DBS investigators; and (3) to maintain a collection of cell lines as a repository.

Methods Employed: Information regarding various techniques and methodology as related to large-scale production of cells is gathered from the literature and by consultation. This information is evaluated and applied in pilot studies to the production of large quantities of cells. Upon successful application, these improved and tested techniques are standardized as part of the Cell Biology Section procedures for mass-cell cultures of high quality for research and vaccine testing purposes.

Major Findings: 1. The Cell Biology Section (CBS), LVR, has provided quality cell cultures to other DBS investigators (see Section of the Laboratory Summary).

2. Upon the recommendation of Dr. Michael F. Barile, Laboratory of Bacterial Products, DBS, his semi-solid Mycoplasma medium without antibiotics and thallium acetate has been added to the three other bacteriological media used to monitor sterility of the tissue culture reagents and of the cell cultures. Barile's mycoplasma medium has indeed detected bacterial infections when the other bacteriological media failed. A total of 1502 lots of cells were prepared with 1.5% bacterial contamination.

3. Mycoplasma testing of all cell lots frozen in the cell repository is now complete and all new lots are frozen and kept after they have been certified as being Mycoplasma-free. This year 49 lots (a total of 1679 ampoules) of Mycoplasma-free cells were frozen in liquid nitrogen. Mycoplasma-monitoring of stock cultures for production purposes is performed under contract weekly.

4. Several cell lines infected with mycoplasma have been successfully cured of the infection by combination treatment with Tylosin and DMSO. While we do not routinely treat mycoplasma infected cells, we developed this method of treatment which can be used in those cases when noninfected cells cannot otherwise be obtained.

5. In conjunction with the Environmental Services Branch, NIH, a modified vertical laminar flow hood to accommodate large-size containers for mass production work was developed. The laminar flow hood has an "elevator" system to adjust the height of tall containers.

6. The acquisition of fetal calf serum certified as being free from bovine viruses and mycoplasma is becoming more difficult due to competition for the product and to the rejection of more lots of serum. Some suppliers no longer have large inventories on hand. However, we are examining serum from several companies who are producing sterile serum by means other than by filtration, such as the "Gray" process, the Flow Laboratory Experimental process, etc.

7. The following cell lines were acquired during the year and are available:

(a) CV-1: African Green monkey kidney
(b) DBS-FRhL-2: Diploid fetal rhesus monkey lung
(c) DBS-FCL-1: Diploid fetal African Green monkey lung
(d) Detroit 510: Galactosemic Human Skin Fibroblast

Significance to Bio-Medical Research and the Program of DBS: In order to assure reproducibility of results obtained by investigators in their control and research studies, standardization of our cell culture methodology and systems is of paramount importance.

Proposed Course of the Project: This effort will be continued during the coming year.

Honors and Awards: Employee Suggestion Award (Peter G. Probst)

Publications:

1. Probst, P. G., Rogers, J. L., and Orr, H. C.: A "Cradle" for support of roller-bottle cell cultures during microscopic examination. Biotech Bioeng. 14: , 1972 (In press).

Serial Nos. 165, 166, 167
1. Virology & Rickettsiology
2. Virology & Rickettsiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Evaluation of the significance and contribution of the cellular immune system in protective immunity and disease

Previous Serial Numbers: Same

Principal Investigator: Daniel E. Thor, M.D., Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:
Total:
Professional:
Other:

Project Description:

This project has been discontinued because of the resignation of the principal investigator on August 31, 1971.

Annual Report of
Laboratory of Viral Immunology
Division of Biologics Standards
July 1, 1971 through June 30, 1972

Laboratory Chief's Summary of Research

The program of the Laboratory of Viral Immunology is research oriented and directed so as to meet current and anticipated needs of the Division in the area of viral diseases. The Laboratory conducts direct research, assists in contract-supported collaborative research management and is involved in the review of data submitted to the Division in its capacity as a regulatory agency. These reviews deal with existing biological products, license applications for new products and information submitted in connection with investigational new drugs (biologics). Laboratory work involves product testing and development of new test procedures as well as more widely ranging investigational efforts. Research conducted by the LVI tends to be applied in nature and product or disease oriented. The conduct of clinical trials is an integral part of the program.

The strength of the Laboratory's operation lies in the bringing together over a period of years of a highly qualified and motivated staff capable of promoting the necessary interaction between regulatory needs and research developments. Senior personnel are familiar with regulatory requirements and manufacturing practices and have product testing and other control responsibilities. These same people direct research and are recognized as experts in their scientific fields. The investigations yield data needed in the regulatory program. Equally important, by virtue of their investigations, our staff is able to maintain lines of communication with other laboratories and to analyse critically information generated by other scientists that may be of current or future consequence in controlling biologics. Our staff received a number of honors during the year. Several served as consultants to expert committees and were invited to participate at international and national conferences. Two LVI investigators received one of America's top research prizes, the Joseph P. Kennedy, Jr. Foundation International Award for Distinguished Scientific Research in recognition of their contributions to the control of rubella.

Selected items from the program are discussed in the following paragraphs.

Hepatitis. Approximately one third of the total effort of the LVI is committed in this area. The work is following two lines: far ranging research on the disease (and viruses causing the disease) and targeted efforts to insure that pertinent new knowledge is immediately translated into the regulatory program to improve the safety of blood and blood-derived products. Human blood, has in the past, often contained hepatitis B (serum hepatitis) virus and recipients were thus at risk of contracting this serious and sometimes fatal disease. Only recently as a result of a substantial research breakthrough has it been possible to develop tests capable of detecting the virus. These tests are complex immunological procedures, yet through the targeted efforts of LVI scientists and other investigators the procedures

have been perfected to the point of having wide application. The DBS has developed standards and rated the sensitivity and reliability of each of the methods. Our staff has been involved in extensive comparative testing with pharmaceutical manufacturers and as a result of this rapid translation of research into the regulatory program, there are now nine manufacturers licensed to produce diagnostic kits for use in blood banks and other laboratories. The use of these new standarized reagents is expected to reduce the incidence of post-transfusion hepatitis caused by this virus by at least 50%. The broader hepatitis research efforts include a search for still better methods of virus detection, a study of the role of antibody and immunity in preventing the disease and an investigation of the importance of this virus in other serious illnesses such as liver cancer, arthritis (and other "auto-immune" diseases) and chronic liver failure. Preliminary work indicates that many persons with the above listed diseases are indeed carriers of the virus. Our hepatitis investigations serve as an example of the multiple approaches taken in solving important problems. There is a major "in house" research committment, a broad collaborative (contract-supported) effort supervised by our scientists and extensive collaboration in testing and exchange of data with pharmaceutical manufacturers and investigators in universities and in other government laboratories. Our personnel have assisted in drafting regulatory requirements, been involved in product testing, developed standards, participated in important research discoveries and served on numerous committees as experts in their field. Their activities have ranged from basic laboratory research to clinically oriented epidemologic studies in hospitals and blood banks of transfusion-associated risks to blood recipients.

Viruses and Cancer. All evidence points to viruses as a major cause of human cancer. In the veterinary field the identification of a herpesvirus as responsible for a common malignancy of chickens has now been followed by the development of a virus vaccine that has effectively prevented these cancers. The implications of these and other recent discoveries in human medicine have been widely appreciated as can be judged by the enormous present and projected committment of public funds to cancer research. Moving with this important field and anticipating the possible future development of new anti-cancer biological products, the LVI has increased its efforts in the herpesvirus and viral oncology area from approximately 10% of total effort four years ago to approximately 30% today. In the current reporting year LVI investigators have shown the genome of EB virus (the herpes virus recovered from African lymphoma tumor tissue) as it exists in a silent state in human white blood cells can be activated to a replicating virus cycle by certain types of drug treatment. Also our scientists have discovered an entirely new family of herpesviruses of subhuman primates that like EB virus appear to associate with the white blood cells of the animal and induce a life-long carrier state. There are many implications of this work, one is that of using subhuman primates and these new viruses as models for the study of herpesviruses and cancer and herpesvirus immunity. In recognition of the quality of the research effort of the laboratory, one member of our staff is a member of the Developmental Research Working Group of the Special Virus Cancer Program of the National Cancer Institute.

Arthritis, Autoimmune Disease and Long-Term Effects of Virus. There is speculation that viruses may be important causes of autoimmune diseases of man. The postulation is that after an initial infection, viral antigens might persist and years later trigger destructive antibody responses against the human tissues harboring the viral antigens. Rheumatoid arthritis could be an example of this situation. It is known that infection with either natural rubella virus or attenuated (vaccine) strains of rubella virus is often associated with viral replication in joint tissues and transient evidence of arthritis. The importance of determining the role, if any, of this virus in progressive arthritic conditions, such as rheumatoid arthritis, is apparent. The LVI has been applying the newer tools of virologic study to arthritis over the past several years and to date has found no evidence of association of rubella with chronic arthritis.

A new section of the laboratory is conducting a long-term study of inactivated influenza vaccine in animals. Here the intent is to determine if over a period of time the protective effect induced by vaccine could wane leaving a hypersensitivity that could enhance susceptibility to influenza. This type of investigation is important since this unexpected and undesirable effect has been noted following immunization with killed measles and killed respiratory syncytial virus vaccines. It should be noted that no adverse effects of this type has been associated with influenza vaccine use. Nevertheless, the initiation of studies searching for unexpected long-term effects of viruses and viral vaccines is extremely important and it is our intent to increase the scope of our long-term investigations.

Clinical Studies. Clinical trials to confirm and extend data aquired by manufacturers are an integral part of our program. Our laboratory has conducted experimental studies with each of the live virus vaccines licensed by the Division since 1960. These include the vaccines for poliomyelitis, measles, mumps and rubella and the new combinations of certain of these vaccines. Normally our clinical trials preceed the development of regulations and license but may include vaccines in routine use if there is a need for additional data. During the current report period LVI medical officers have conducted investigations with all four of the above listed live virus vaccines to obtain information relating to potency ranges and duration of vaccine-induced immunity.

As part of this work the laboratory developed two new serologic procedures to measure polio and mumps antibody levels (immunity). These techniques employ microtiter methods and as a result require only tiny amounts of human serum, a distinct advantage in measuring the antibody response of infants and preschool children.

Cell Culture Studies. Cell cultures are basic to virtually all research with viruses; consequently many projects in the laboratory involve cell experimentation. During the current year both intramural and collaborative research efforts have been continued on several cell substrates used for vaccine production: monkey kidney, rabbit kidney, duck embryo and human diploid fibroblasts. These studies have increased our knowledge of the chromosomal and other characteristics of these cells and on their freedom from

contamination by infectious agents indigenous to the host source. The laboratory has prepared and used more than 30 distinct types of tissue cells from man, monkeys, rabbits, dogs, mice, rats, swine and hamsters in its research program. These included cells derived from lung, kidney, skin, muscle, joint surfaces, cartilage, bone marrow, blood, liver, spleen, eye, salivary gland and prostate gland. The LVI maintains many of the special cells as permanent reference cultures frozen in liquid nitrogen. These cultures are furnished on request to investigators all over the world.

Special Problems. There are two special problems that should be mentioned since they have an major hearing on current and future performance of the organization: These are: (1) an imbalance between needs and resources (including personnel strength) to meet those needs and (2) our susceptibility to greater damage than benefit as a result of the continuing critical dialogue concerning the performance of the DBS as a regulatory organization. As I see it our most critical needs in the coming year will be in trying to find ways to correct these problems.

LABORATORY OF VIRAL IMMUNOLOGY

Control Program

July 1, 1971 through June 30, 1972

Antibody Assays: Using virus neutralization or hemagglutination-inhibition tests.

Immune Serum Globulin (Human)

Number of lots received	195
Poliovirus potency tests	205
Number of tissue cultures	43,050
Measles potency tests (HAI)	247
Number of titrations	741

Rh_o (D) Immune Globulin (Human)

Number of lots received	41
Polio tests	42
Number of tissue cultures	8,820

Testing of Reference Materials:

NIH Measles Reference Serum No. 1

Number of tests (HAI)	19
Number of titrations	57

NIH Reference Poliomyelitis Immune Globulin Lot 174

Number of tests for polio antibodies	40
Number of tissue cultures	8,400

NIH Reference Poliomyelitis Immune Globulin Lot 175

Measles HAI tests	40
Number of titrations	120

Poliomyelitis Reference Serum IIA-4

Number of tests	24
Number of tissue cultures	6,000

Testing of Vaccines, Live, Attenuated:

Poliovirus, Oral (Screening of pre-inoculation monkey sera)

Number of neurovirulence tests	21
Number of sera	735
Number of tissue cultures	5,145

Testing of Vaccines, Inactivated:

Poliomyelitis

Number of lots received	6
Monkey potency tests	4
Pre-inoculation sera	48
Post-inoculation sera	48
Number of tissue cultures	7,056

Potency and Marker Tests (Live, Attenuated Vaccines);

Testing of Reference Materials:

A. NIH Poliovirus Reference Preparations TA_3, TB_3, TC_3

1. Tests for Virus Content

a. Number of tests	34
b. Number of tissue cultures	1,224

B. NIH Poliovirus Reference Preparations Neurovirulent

1. Tests for Virus Content

a. Number of tests	15
b. Number of tissue cultures	90

Testing of Vaccines, Live, Attenuated:

1. Poliovirus

a. Monovalent lots tested for	
(1) Virus Content	9
(2) Genetic Marker	24
b. Trivalent lots tested for	
(1) Virus Content	29
c. Number of tissue cultures	2,412

2. Measles

Vaccine lots tested	27
Number titrations	85
Number of tissue cultures	2,720
DBS Reference	
Number titrations	42
Number of tissue cultures	2,720

3. Mumps

Vaccine lots tested	19
Number titrations	75
Number of tissue cultures	2,400
DBS Reference	
Number titrations	36
Number of tissue cultures	1,152

4. Rubella

Vaccine lots tested	17
Number of tissue cultures	3,875

5. Combined Mumps - Rubella

Vaccine lots tested		1
Mumps	2 titrations	64 tissue cultures
Rubella	2 titrations	50 tissue cultures

6. Combined Measles - Rubella

Vaccine lots tested		3
Measles	12 titrations	384 tissue cultures
Rubella	8 titrations	200 tissue cultures

7. Combined Measles - Mumps - Rubella

Vaccine lots tested		2
Measles	5 titrations	160 tissue cultures
Mumps	5 titrations	160 tissue cultures
Rubella	5 titrations	125 tissue cultures

Titration of DBS Rubella Virus Reference Lot 1

Number of tests	41
Number of tissue cultures	1,890

Potency Tests - Hepatitis Associated Antibody (Anti-Australia Antigen)

Total Lots tested	165
a. Counterelectrophoresis	142
b. Agar Gel Diffusion	16
c. Complement Fixation	3
d. Rheophoresis	1
e. Radioimmunoassay	11
f. Latex particle agglutination	1

Assay of Sera For Antibodies

1. Poliovirus I, II, III

Human	193
No. of tissue cultures	16,212

2. Rubella HI

Human	2,023
Guinea Pig	97
Immune Globulin (H)	193

3. Measles HI

Human	1,455
Monkey	23

4. a. Mumps - Plaque Reduction

Human	8
Control (guinea pig, human)	14
Immune Globulin	2
No. of tissue cultures	120

b. Mumps - Tissue Culture Neutralization (Microtiter)

Human	489
Control (guinea pig, human)	47
Immune Globulin	1

c. Mumps - HI

Human	277
Control (guinea pig, human)	12

LABORATORY OF VIRAL IMMUNOLOGY

Production of Cell Cultures for Use in Control and Research Activities

July 1, 1971 through June 30, 1972

	Tubes	2 oz	8 oz	32 oz	Petri Dishes	MT Plates
Simian:						
BS-C-1	220	436	22	4		
VERO	400	308	176	47	126	2803
FRhL-2	647	360	72	63	196	48
FRhL-4		280	16		10	2
FCL-1						
FCL-2		133			30	
MRC-5		116				1
ES-1		116				1
WBC:						
Rhesus		30	30			
Chimpanzee		30	30			
Human:						
Bone Marrow (BoMa)	40	1100		55		
" " (D-6)		40	150			
Fetal Liver		280		6		
Synovium	500	70	80			1
Lung		40	35	1		
Prostate		40	150	2		10
Skin-Muscle		50	60	2		
Leukocytes		200				
Rabbit:						
Skin	600		40	10		
Cornea	300		20	5		
Spleen	300	198	20	5		
Liver	300	304	25	5		
Kidney		356	10			
Salivary Gland		96				
Misc:						
Pig Synovium		248	150			
BHK-21		104				

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LABORATORY OF VIRAL IMMUNOLOGY

Cell Lines Prepared and Stored in Liquid N_2

Total Number:

Cee Lines (Tissue)	No. Lines	No. Amps.
Human Leukocytes	9	36
synovium	18	72
fetal liver	8	32
bone marrow	2	10
diploid fibroblast	1	6
diploid fetal lung	1	4
prostate	1	4
skin muscles	1	4
lung	1	4
cartilage	1	4
Rabbit spleen	4	16
liver	7	28
kidney	8	32
salivary gland	2	8
Monkey-fetal rhesus	1	4
" " kidney	1	4
" " lung	2	16
cercopithecus kidney	2	9
fetal cercopithecus lung	2	12
Rat fibroblast	1	4
Mouse epithelial	1	4
Dog synovium	1	4

Serial No. DBS 49
1. Laboratory of Viral Immunology
2. Section on General Virology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Investigations concerning rubella virus and vaccine

Principal Investigator: Paul D. Parkman, M.D.

Other Investigators: Hope E. Hopps, M.S.
Joan C. Enterline, B.A.
Eva R. Brown, B.S.
MacGregor Conliffe, B.S.
Rudyard Wallace
Harry M. Meyer, Jr., M.D.

Cooperating Units: None

Man Years:

Total:	2.3
Professional:	1.4
Other:	0.9

Project Description: Studies of rubella virus infection in man.

Objectives: To evaluate the effects of infection with natural or attenuated rubella viruses.

Methods Employed: Standard assays for isolation of rubella virus, markers of rubella virus attenuation and rubella antibodies were employed.

Major Findings: Despite the fact that virological and serological methods for rubella diagnosis have been available for more than a decade, many laboratories still experience difficulty in performing such studies. Specimens from a total of 6 subjects were examined in some detail. These included 3 patients suspected to have congenital rubella syndrome defects and 4 women exposed to rubella virus or vaccinated during pregnancy. In one instance rubella virus was recovered from multiple fetal tissues obtained at therapeutic abortion of a woman inadvertently vaccinated during pregnancy. Marker tests to determine if these isolates have the marker characteristics of the vaccine strain are in progress.

Serological testing of specimens from rubella vaccines immunized during the period from 1965 to 1969 at the Arkansas Children's Colony continues to provide information on the long-term persistance of vaccine induced

antibodies and the responses of immunized children exposed to natural rubella infection. Thus far results indicate that vaccines are afforded complete protection against the natural disease for as long as 6 years after vaccination. No evidence of clinical rubella has been observed in immunized children exposed to cases of natural rubella whether or not anamnestic antibody responses occurred after contact.

Significance to DBS Program and Bio-Medical Research: All work pertains to problems concerned with the prevention of rubella and the control of rubella vaccines.

Proposed Course of Project: This project will be continued.

Honors and Awards: Joseph P. Kennedy, Jr. Foundation International Award for Distinguished Scientific Research, 1971.

Publications:

Parkman, P. D., Hopps, H. E. and Meyer, H. M., Jr. Rev. Medecine, No. 22, p. 1375-1386, May 31, 1971.

Meyer, H. M. Jr. and Parkman, P. D.: Rubella Vaccines. Proceedings of the 8th Annual Immunization Conference, Kansas City, Mo., March 2-4, 1971, U. S. Department of Health, Education and Welfare Publication, p. 32-33.

Serial No. DBS 127
1. Laboratory of Viral Immunology
2. Section on Viral Genetics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Studies of Viruses Associated with Infectious Mononucleosis and Human Lymphoma.

Previous Serial Number: 127

Principal Investigator: Paul Gerber, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total: 2
Professional: 1
Other: 1

Project Description: Our studies concentrated on the relationship of Epstein-Barr virus (EBV) and human lymphocytes.

Major Findings: (1) The EBV genome present in most continuous human lymphoblastoid cell lines, could be activated by treatment with 5-Brdu. This appears to be the first demonstration of *in vitro* activation of a latent herpes virus of man. (2) Lymphocytes obtained from persons with previous EBV infection(s) could be stimulated *in vitro* by UV-inactivated EBV. This reaction is immunologically specific and may be useful in studies of cell-medicated immunity in EBV infection. This method should also be applicable to other viruses.

Significance to DBS Program and Bio-Medical Research: Studies concerned with the detection of viruses in apparently "virus-free" cells are of importance to the program of this Division.

Honors and Awards:

Member of Developmental Research Working Group of the Special Virus Cancer Program, NCI

Invited speaker and rapportem at the Symposium on "Oncogenesis and Herpes-Type Viruses" Christ's College, Cambridge, U.K. June 20-25, 1971.

Invited speaker at the International Symposium on Comparative Leukemia Research, Padua, Italy. September 13-17, 1971.

Invited speaker, International Symposium on Hodgkin's Disease, Stanford University, March 20-25, 1972.

Invited speaker by National Foundation Conference on "Application of Long Term Lymphocyte Cultures to Human Studies," Airlie House, March 27, 1972.

Publications:

Gerber, P. and Hoyer, B. H. Induction of Cellular DNA Synthesis in Human Leukocytes by Epstein-Barr Virus. Nature 231: 46-47, 1971.

Deal, D. R., Gerber, P. and Chisari, F. V. Heterotransplantation of Two Human Lymphoid Cell Lines Transformed in vitro by Epstein-Barr Virus. J. Nat. Canc. Inst. 47: 771-780, 1971.

Gerber, P. Activation of Epstein-Barr Virus by 5-Bromodeoxyuridine in "virus-free" Human Cells. Proc. Nat. Acad. Sc. 69: 83-85, 1972.

Serial No. DBS 47
1. Laboratory of Viral Immunology
2. Section on Viral Biology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Investigations Concerning the Smallpox Group of Viruses and Smallpox Vaccines.

Previous Serial Number: DBS 47

Principal Investigator: Ruth C. Dunlap, Ph.D.

Other Investigators: Lewellys F. Barker, M.D.
G. D. Washington, B.A.
Joseph A. Markenson, M.D.
H. M. Meyer, Jr., M.D.

Cooperating Units: None

Man Years:

Total:	2.2
Professional:	1.2
Other:	1.0

Project Description: Part I. Basic Studies of Vaccinia Viruses.

A. Reactivation of irradiated vaccinia virus in irradiated MA-104 cells. MA-104 cells were irradiated with UV light for varying lengths of time from 0 to 35 sec at an intensity of 4.2 ergs/mm^2/sec. Vaccinia virus (CV1-80) was irradiated 0, 15, 30, 60 or 90 sec at an intensity of 6.4 ergs/mm^2/sec. The irradiated virus was titrated on each set of irradiated cells. In cells irradiated for 5-15 sec, irradiated virus titers were slightly higher than in cells which were not irradiated. This increase was most marked with virus irradiated for 60 or 90 sec. The maximum increase in titer was about 3-fold. The hypothesis involved is that UV repair mechanisms initiated to repair cellular DNA damage can also be utilized to repair damaged viral DNA.

B. Studies of CV1-78 vaccinia in human embryo kidney (HEK) cells. The CV1 strain of vaccinia displays inhibition of replication in HEK cells, although other strains of vaccinia grow well in this substrate. Ultrathin sections of HEK cells infected either with calf lymph vaccinia virus (CL) or CV1 vaccinia have been prepared from cultures harvested 4, 8 and 12 hours after infection. This study is still in the preliminary stages

due to the complexities of training personnel in this exacting procedure. The work will be continued and it is hoped that the stage of development at which CV1 is blocked in HEK cells can be demonstrated photographically.

C. Solid phase radioimmunoassay for detection of anti-vaccinial antibody. This project has been initiated in hopes of providing a model for improved immunological quantitation. Tubes are coated with a known quantity of vaccinia virus, determined by electron microscopic particle count. An anti-vaccinial antibody preparation is added to the tubes, followed by the addition of labelled purified vaccina, also carefully quantitated. The amount of antigen-antibody-labelled antigen binding is measured in the gamma counter.

Project Description: Part II. Clinical Studies.

There have been no clinical studies under this project this year.

Significance to DBS Program and Bio-Medical Research: This work pertains to a better understanding of the properties of vaccinia virus strains used as vaccines in the prevention of smallpox.

Proposed Course of the Project: This project will be continued with emphasis on differing characteristics of vaccinia strains used or considered for use as anti-smallpox vaccines.

Honors and Awards: None

Publications: None

Serial No. DBS 48
1. Laboratory of Viral Immunology
2. Section on Immunology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Investigations concerning mumps virus and vaccine

Previous Serial No.: DBS 48

Principal Investigator: Hope E. Hopps, M.S.

Other Investigators: Barbara C. Meyer, M.A.
Robert D. Douglas
Michael S. Williams
Harry M. Meyer, Jr., M.D.

Cooperating Units: None

Man Years:

Total:	1.1
Professional:	0.7
Other:	0.4

Project Description: Development of a new test system for measuring mumps antibody levels.

During the past year a microtiter test system has been developed for measuring mumps neutralizing antibody; the test is performed with the stable Cereopithecus kidney cell line (Vero) using 2 fold sereal dilution of test sera and a constant dose of virus (Sporn strain, Vero-cell adapted). Cytopathic change is easily read and the serum titers are calenlated after a 7 day reading. The test is equally as sensitive as the Vero cell plaque inhibition test formerly used but can be performed with greater technical ease and is more economical. A four year survey of antibody stability in individuals at the Arkansas Children's Colony who received the Jeryl Lynn strain mumps vaccine or who sustained natural mumps injection during the period in which the vaccine was being administered will be performed in the new test system. Annual surveys of these populations will continue. Studies concerning development of mumps antibody in individuals receiving combined vaccines are reported under Project Report No. DBS 126.

The potency testing of mumps vaccine or combined vaccines containing mumps virus are reported under Summary of Control Activities.

Proposed Course of Project: These studies will be continued.

Honors and Awards: None

Publications: None

Serial No. DBS 46
1. Laboratory of Viral Immunology
2. Section on Immunology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Investigations concerning measles virus and vaccine

Previous Serial Number: DBS 46

Principal Investigator: Barbara C. Meyer, M.A.

Other Investigators: Hope E. Hopps, M.S.
Harry M. Meyer, Jr., M.D.

Cooperating Units: None

Man years:

Total: 1.3
Professional: 0.7
Other: 0.6

Project Description: Investigations concerning the efficacy of vaccines comprising measles-mumps-rubella, measles-rubella and mumps-rubella were undertaken. See Project Report No. DBS 126. Vaccine lots tested are reported under summary of Control Activities.

Honors and Awards: None

Publications: None

Serial No. DBS 53
1. Laboratory of Viral Immunology
2. Cell Metabolism Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Study and diagnosis of heritable metabolic diseases by use of cells cultured in vitro.

Previous Serial Number: DBS 53

Principal Investigator: B. Wm. Uhlendorf, Ph.D.

Other Investigators:
S. Harvey Mudd, M.D.
Elizabeth Neufeld, Ph.D.
J. Charles Pallavicini, Ph.D.
Roscoe O. Brady, M.D.
Jeffrey R. Poole, M.D.
Cecil B. Jacobson, M.D.
John M. Freeman, M.D.
Vivian E. Shih, M.D.

Cooperating Units:
Laboratory of General Comparative Biochemistry, NIMH
Laboratory of Biochemistry and Metabolism, NIAMD
Pediatric Metabolism Branch, NIAMD
Laboratory of Neurochemistry, NINDS
Digestive and Hereditary Disease Branch, NIAMD
Department of OB-GYN, George Washington University Medical Center
Department of Neurology, Johns Hopkins Hospital
Neurology Service, Massachusetts General Hospital

Man Years:

Total:	1.14
Professional:	1.0
Other:	0.14

Project Description: The concept which is the basis of the subprojects listed below is that a heritable metabolic disorder of an individual's metabolism may be reproduced in cultures of his cells.

Objectives and Major Findings: (1) Cystic fibrosis (CF): Continued experiments with cultured CF fibroblasts have tended to rule out an electrolyte pump blocking factor as being responsible for elevated glycogen levels in CF cells. I am no longer actively participating in the project except as a consultant.

(2) Homocystinuria: We have described another new type of homocystinuria. Cultivated fibroblasts from patients with this type of homocystinuria exhibit normal levels of cystathionine synthase (deficient in the classical type of the disease) and normal levels of N^5-methyltetrahydrofolate-homocysteine methyltransferase (deficient in another type of the disease but markedly low levels of methylenetetrahydrafolate reductase. A deficiency of this reductase activity can explain the biochemical abnormalities of these patients.

Our studies of the classical cystathionine synthase deficiency type of homocystinuria suggest that the pyridoxine-responsive and the pyridoxine-nonresponsive forms of the disease are genetically as well as biochemically distinct. We are exloring this possibility by cell hybridization experiments.

(3) Prenatal diagnosis: By making use of cultured amniotic fluid cells we have made prenatal diagnoses of the Hurler and Hunter diseases, of Niemann-Pick disease and of Fabry's disease.

Significance to DBS Program and Bio-Medical Research: Human diploid cell culture is a powerful tool for the early prenatal diagnosis of heritable metabolic diseases and for studies of their underlying biochemical defects.

Proposed Course of Project: This is a continuing project.

Honors and Awards: None

Publications:

Mudd, S. H., Uhlendorf, B. W., Freeman, J. M., Finkelstein, J.D. and Shih, V. F.: Homocystinuria associated with decreased methylenetetrahydrofolate reductase activity. Biochem. Biophys. Res. Comm. 46: 905-912, 1972.

Uhlendorf, B. W.: Use of amniotic fluid and reliability of diagnostic procedures. In HEW publication No. (NIH) 72-25, Harris, Maureen (Ed.): Early diagnosis of human genetic defects: scientific and ethical considerations. U. S. Government Printing Office, Washington, D. C., pp. 149-168, 1971.

Brady, R. O., Johnson, W. G., and Uhlendorf, B. W.: Identification of heterozygous carriers of lipid storage diseases. Amer. J. Med. 51: 423-431, 1971.

Blass, J. P., Lonsdale, D., Uhlendorf, B. W., and Hom, E.: Intermittent ataxia with pyruvate-decarboxylase deficiency. Lancet 1: 1302, 1971.

Goodmann, S. I., Moe, P., Hammond, K. B., Mudd, S. H., and Uhlendorf, B. W.: Homocystinuria with methylmalonicaciduria: two cases in a sibship. Biochem. Med. 4: 500-515, 1970.

Mahoney, M. J., Rosenberg, L. E., Mudd, S. H., and Uhlendorf, B. W.: Defective metabolism of vitamin B_{12} in fibroblasts from children with methylmalonicaciduria. Biochem. Biophys. Res. Comm. 44: 375-381, 1971.

Serial No. DBS 137
1. Laboratory of Viral Immunology
2. Section on Viral Biology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Investigations into the Etiologic Agents of Viral Hepatitis

Previous Serial Number: DBS 137

Principal Investigator: Lewellys F. Barker, M.D.

Other Investigators:
Robert J. Gerety, M.D.
Joseph A. Markenson, M.D.
John Thiel
Hope E. Hopps, M.S.
Harry M. Meyer, Jr., M.D.
Roderick Murray, M.D.
Ruth L. Kirschstein, M.D.
Douglas E. Lorenz, Ph.D.
Francis V. Chisari, M.D.
Philip P. McGrath
Edward B. Seligmann, Jr., Ph.D.
Robert H. Purcell, M.D.
N. Raphael Shulman, M.D.
D. Gordon Sharp, Ph.D.
Martin Goldfield, M.D.
Dan W. Dalgard, D.V.M.
Clarence J. Gibbs, Ph.D.
Robert Cornelius, D.V.M.
Charles L. Vogel, M.D.
Bruce L. Smith, M.D.
S. K. Sama, M.D.
Ian Mackay, M.D.

Cooperating Units:
Office of the Director, DBS, NIH
Laboratory of Pathology, DBS, NIH
Laboratory of Control Activities, DBS, NIH
Hematology Branch, National Institute of Arthritis and Metabolic Diseases, NIH
Laboratory of Blood and Blood Products, DBS, NIH
Laboratory of Infectious Diseases, NIAID, NIH
Department of Bacteriology and Immunology, University of North Carolina School of Medicine, Chapel Hill, N.C.
Division of Laboratories, State of New Jersey Department of Health, Trenton, N.J.

Collaborative and Field Research Unit, NINDB, NIH
Hazleton Laboratories, Vienna, Va.
Uganda Cancer Institute, Kampala, Uganda

Man Years:

Total:	7.4
Professional:	3.4
Other:	4.0

Project Description: Part I. Evaluation of the role in acute and chronic viral hepatitis and chronic liver disease of the hepatitis B antigen (HB Ag) and the relationship of HB Ag to hepatitis B virus (HBV).

Characterization and clinical epidemiologic and immunologic studies of HB Ag have been conducted. Attempts to transmit HBV to experimental animals and cell cultures are continuing.

Project Description: Part II. Search for other agents responsible for viral hepatitis, type A and chronic hepatitis.

Epidemiologic and virologic studies of viral hepatitis, type A, are continuing.

Objectives: The goal of these studies is the isolation and identification of viruses which produce hepatitis in man and the eventual control of viral hepatitis, types A and B, by blood donor screening and passive and active immunization.

Methods Employed: Standard epidemiologic, serologic and virologic techniques are used throughout this project.

Major Findings: Part I.

A. Standards and Potency Testing of Hepatitis Associated Antibody (Anti-Australia Antigen):

Potency and specificity testing of Hepatitis Associated Antibody (Anti-Australia Antigen) pre and post-licensing (9 companies have been licensed to produce this product) is done in LVI (see section on Control Activities). A new reference panel for control testing purposes has been selected and prepared under the joint supervision of LCA and LVI.

Efforts to improve the sensitivity and practicability of tests for HB Ag and for anti-HB Ag and to devise standards to evaluate new tests and reagents are continuing. Major emphasis during the past year has been placed on radioimmunoassay, passive hemagglutination and latex particle agglutination methods.

B. Serologic Studies:

Comparison of counterelectrophoresis (CEP), complement fixation (CF), latex particle agglutination (LPA) and radioimmunoassay (RIA) procedures for HB Ag

detection has been conducted during the past year. RIA would appear at present to provide the greatest sensitivity of the various immunologic procedures, but the exquisite sensitivity of RIA methods is accompanied by specificity and reproducibility problems which have not been resolved.

CEP, RIA and passive hemagglutination (PHA) methods are being evaluated for antibody detection. The latter methods are quite sensitive but specific inhibition of reactions with HB Ag is necessary to demonstrate specificity.

The AD and AY serotypes of HB Ag are being determined by agar gel diffusion (AGD) and CEP, using discriminating antisera prepared by hyperimmunization of guinea pigs with purified subtype antigens. New subtype determinants and the application of more sensitive techniques such as radioimmunoassay for subtype determination are being evaluated. Subtypes of reference panel antigens have been determined. Correlation of subtypes with clinical manifestations represented in various serum collections are under study. Solid phase immunoadsorption methods are being developed for purification of antibodies. Means of detecting HB Ag in the presence of anti-HB Ag excess or in Ag-Ab complexes are being studied with the objective of enhanced HB Ag detection and evaluation of the significance of immune complexes.

C. Clinical-Epidemiologic Studies:

Studies of HB Ag and anti-HB Ag in acute hepatitis and chronic liver diseases, particularly hepatoma and cirrhosis, are continuing in East Africa. A very striking association between HB Ag persistence, presumably indicative of the HBV carrier state, has been further documented in hepatoma and macro-nodular or post-necrotic cirrhosis. The addition of RIA screening has increased the detection rate of HB Ag in this and other populations quite significantly; anti-HB Ag detection has been primarily by PHA and demonstrates a high endemicity of HBV infection in East Africa. Further studies of HB Ag in hepatitis cases which occurred in the 1951 to 1954 studies using RIA methods are in progress. A serologic study of the role of HBV in chronic active hepatitis in Australia has been initiated.

D. Electron Microscopic Studies:

1) Thin section studies have been done in LP of liver biopsies from infected chimpanzees. The presumed core particles of HBV have been found in the nuclei of cells during the acute experimental infection, but not in a chronically infected animal. Extension of these electron microscopic studies to examination of inoculated cell cultures has been initiated.

2) Examination of HB Ag particles in multiple sera has been initiated with LP in search of one or more sera that are rich in the large, 40-42 nm, "Dane" particles which are thought to consist of the core surrounded by an outer HB Ag coat. The eventual objective of these studies is the preparation of a purified concentrated "Dane" particle preparation in order to conduct immunologic and virologic studies of the cores.

E. Primate Studies:

Two chimpanzees free of detectable HB Ag and anti-HB Ag were inoculated with one ml of the NIH icterogenic plasma pool. One showed no evidence of acute hepatitis, but developed a rising titer of anti-HB Ag 13 weeks post-inoculation. The second animal became sero-positive for HB Ag 15 weeks post-inoculation. HB Ag remained detectable for 14 weeks, after which anti-HB Ag appeared. Nine weeks after HB Ag became detectable, the serum SGPT level rose and remained elevated for 9 weeks. Plasma with an HB Ag CF titer of 1:256, collected from this animal, was inoculated into two more antibody-free chimpanzees. Neither developed biochemical or histologic evidence of acute hepatitis, but both became sero-positive for HB Ag 6 weeks post-inoculation and then developed anti-HB Ag. Inoculation of HB Ag partially purified by isopycnic banding in a continuous sucrose gradient was followed by serum HB Ag appearance in one animal. These experiments have demonstrated the value of the chimpanzee for experimental studies of the transmission and pathogenesis of viral hepatitis, type B and these studies are continuing.

In addition, attempts to transmit viral hepatitis, type B, to a variety of smaller primates including gibbons, rhesus monkeys, patas monkeys, vervet monkeys, squirrel monkeys, owl monkeys and cebus monkeys are in progress. The only success to date has been in infant rhesus monkeys. Titrations have been initiated to compare the sensitivity of chimpanzees and rhesus monkeys to HBV infection and studies are in progress to evaluate the effect of immuno-suppression and experimental infection.

F. Tissue Culture Studies:

Attempts are continuing to find a cell culture system which will support replication of HBV in vitro. Current emphasis is on evaluation of inoculated cells by RIA, immunofluorescence and electron microscopy. With the exception of explants from the liver of a chronically infected animal, we have not been able to obtain any evidence of HBV in culture to date.

G. Active and Passive Immunization:

Studies have been initiated on the antigenicity of purified HB Ag for evoking both humoral and cellular immune responses in laboratory animals. Methods for potency testing of hyperimmune globulin directed against HB Ag are under investigation. It is anticipated that studies to evaluate the safety and efficacy of passive and active immunizing materials in experimental animals will be initiated in the coming year.

Major Findings: Part II.

Studies are continuing with LP to try to resolve the question of marmoset susceptibility to viral hepatitis, type A. In addition, we have initiated efforts to transmit the hepatitis A virus to other South American monkeys and are continuing exploratory studies on the propagation of this agent in cell culture.

Significance to DBS Program and Bio-Medical Research: Viral hepatitis, types A and B, are two of the major infectious disease problem areas in modern public health; the work done under this project with the eventual goals of recognizing and understanding the etiologic agents of these diseases and devising methods for their control is highly pertinent to the goals of the DBS.

Proposed Course of the Project: This project will be continued.

Honors and Awards:

Dr. L. F. Barker:

1) Consultant to Ad Hoc Committee on Hepatitis-Associated Antigen (HAA) Test of the Committee on Plasma and Plasma Substitutes, Division of Medical Sciences, National Academy of Sciences - National Research Council.

2) Consultant to the hepatitis program of the National Blood Resources Branch, National Heart and Lung Institute.

3) Consultant to the American Hospital Associations Committee on Infections within Hospitals, Chicago, December 7, 1971.

4) Participant in Ad Hoc Conference on Safety Recommendations for Laboratory Workers Exposed to Hepatitis Associated Antigen, Center for Disease Control, Atlanta, March 15, 1972.

5) Invited speaker at the Canadian Hepatic Foundation 1971 International Symposium on Viral Hepatitis, Toronto, May 30, 1971.

6) Invited to speak at the 12th International Congress of Microbiological Standardization, Annecy, September 20, 1971.

7) Invited speaker and session chairman at Study Group on Australia Antigen and Seminar on Infectious Hepatitis, New Delhi, October 19, 1971.

8) Invited speaker and session chairman at Australia Antigen Symposium, Philadelphia, November 8, 1971.

9) Invited speaker at Symposium on Viral Hepatitis and Blood Transfusion, San Francisco, March 25, 1972.

Publications:

Barker, L. F. and Gibson, S. T.: Australia antigen ecology. Ann. Intern. Med. 75: 130-132, 1971.

Barker, L. F. and Murray R.: Acquisition of hepatitis-associated antigen. Clinical features in young adults. J.A.M.A. 216: 1970-1976, 1971.

Barker, L. F. and Murray, R.: Relationship of virus dose to incubation time of clinical hepatitis and time of appearance of hepatitis-associated antigen. Amer. J. Med. Sci. 263: 27-33, 1972.

Barker, L. F. and Peterson, M. R.: Detection of hepatitis associated antigen and antibody - Comparison of methods and their application. Canad. Med. Asso. J. 106 (special issue) 486-490, 1972.

Anthony, P. P., Vogel, C. L. Sadikali, F., Barker, L. F., and Peterson, M. R.: Histopathology of cirrhosis. Brit. Med. J., 1972.

Vogel, C. L., Anthony, P. P., Sadikali, F., Barker, L. F. and Peterson, M. R.: Hepatitis associated antigen and antibody in hepatocellular carcinoma: Results of a continuing study. J. Natl. Cancer Inst., in press.

Serial No. DBS 126
1. Laboratory of Viral Immunology
2. Section on General Virology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Investigations concerning combined live virus vaccines.

Previous Serial Number: DBS 126

Principal Investigator: Harry M. Meyer, Jr., M.D.

Other Investigators: Hope E. Hopps, M.S.
Barbara C. Meyer, M.A.
Paul D. Parkman, M.D.
Richard D. Krugman, M.D.
Joan C. Enterline, A.B.
Eva R. Brown, B.S.

Cooperating Units: Center for Disease Control,
Virology Section and Immunization Branch,
Ungulate Unit, NIH Animal Center

Man Years:

Total:	4.1
Professional:	3.2
Other:	0.9

Project Description: Clinical trials of combined and simultaneously administered live virus vaccines.

One trial in progress at the time of the last report has been completed. Children between 1 and 6 years of age residing on the island of Tol in the Truck lagoon received combined live measles and rubella virus vaccines. No significant adverse reactions were noted as a result of immunization. In the group, 197 had no immunity to either measles or rubella prior to immunization (as shown by HI antibody tests). Seroconversion to the measles or component of the vaccine occurred in 96% (190/197) and to the rubella component in 93% (184/197). An additional 129 children possessed antibodies for either measles or rubella (but not both) prior to vaccination and in this group seroconversion rates were also in excess of 90%. The geometric mean titers of antibodies evoked were comparable in both the single negative and double negative vaccinees. These titers were also similar to those obtained in earlier trials using monovalent vaccines for either measles or rubella. Serologic testing of mothers of many of these children identified 10 without rubella antibodies and 2 without measles antibodies. These "susceptible" mothers did develop antibodies as a result of their exposure

to vaccinated children. These data were consistent with information collected earlier by the manufactures and were interperted as indicating that the clinical and immunologic response to combined measles-rubella vaccine was similar to that observed with use of the monovalent components.

Another trial in progress at the time of the last report has also been completed. Here children between 1 and 6 years of age residing on the island of Guam were immunized with a combination of live measles-mumps-rubella vaccine given alone by injection or by this combination given by injection and at the same time an oral feeding of trivalent live poliovirus vaccine. Again the clinical reactions and antibody responses evoked were comparable to those obtained by use of monovalent vaccines. The number of children included in this study was 153 for Measles-Mumps-Rubella vaccine alone and 39 for Measles-Mumps-Rubella given simultaneously with trivalent poliovirus vaccine.

Another trial on the Island of Guam is still in progress. Here children are given oral feedings of trivalent poliovirus vaccine at age 2 months, 4 months and 1 year. During and after this three-inoculation sequence, serum specimens are obtained to determine seroconversion rates and geometric mean titers of antibodies. Approximately 250 children have been enrolled in the study and have now received one or more of the oral feedings of trivalent poliovirus vaccine. Serologic testing of specimens collected will begin in the next few months and continue throughout the next year as increasing numbers of children complete the immunization sequence. It is anticipated that the results of this study will provide new data on dose-effectiveness of polio vaccines in children in this age group. This information should be particularly valuable since most of the polio vaccine used in the United States is of the trivalent type and since most vaccine is used in children in the age group under study.

Significance to the Program of DBS and Bio-Medical Research: All of this work pertains directly to problems in the prevention and control of human infections.

Proposed Course of Project: This project will be continued.

Honors and Awards: None

Publications:

Meyer, H. M., Jr.: Combined Live Virus Vaccines. Proceedings of the 8th Annual Immunization Conference, Kansas City, Mo. U.S. Department of Health, Education and Welfare Publication, p. 61, March 2-4, 1971.

Serial No. DBS 171
1. Laboratory of Viral Immunology
2. Section on General Virology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Investigations concerning cytomegaloviruses and other primate herpesviruses.

Previous Serial Number: DBS 171

Principal Investigator: Paul D. Parkman, M.D.

Other Investigators:
John Bissell, M.D.
Arthur Frank, M.D.
Reed Dunnick, M.D.
MacGregor Conliffe, B.S.
Ronald Mayner, B.S.
Rudyard Wallace
Hope Hopps, M.S.
Barbara Meyer, M.A.
Michael Williams
Richard Krugman, M.D.
Robert Douglas
Harry Meyer, Jr., M.D.

Cooperating Units: None

Man Years:

Total:	7.8
Professional:	6.0
Other:	1.8

Project Description: Isolation and characterization in vivo and in vitro of cytomegaloviruses and other herpesviruses of primates.

Objectives: To evaluate the relationships of herpesviruses of primates and the effects of infection with these agents.

Methods Employed: Virus recovery attempts were performed using cocultivation of leukocytes with fibroblastic cultures of human or simian origin as well as by standard methods.

Major Findings: In the course of investigating the characteristics of cytomegalovirus infections of rhesus monkeys an apparently new herpesvirus was isolated. This virus was recovered from leukocytes and has been termed the rhesus leukocyte associated herpesvirus (LAHV). The majority of animals appear to be infected in nature early in life. The virus appears to persist for years following infection, perhaps for the life of the animal. Experimentally induced infections are subclinical, and are not accompanied by specific hematologic changes. The virus has been shown to have the chemical and physical characteristics of the herpes group of agents; it appears to have patterns of growth similar to the cytomegaloviruses, and is not pathogenic for small laboratory animals or embryonated eggs. Thus far, it has not been shown to be related to known herpesviruses including herpesvirus hominis, tamarinus, simiae or samiri, cytomegaloviruses of human, rhesus and cercopithecus origin, the Liverpool vervet virus described by McCarthy, or varicella-zoster and EB-virus. Studies are in progress concerning the immediate and long term effects of LAHV infection in a variety of primate species.

Significance to DBS Program and Bio-Medical Research: The study of simian agents relates directly to the mission of the Division in biologics control. The study of latent or chronic viral infections is of current interest to those concerned with slow viruses and chronic disease and with oncology.

Proposed Course of the Project: Studies concerning the relationship between cytomagloviruses, the LAHV strains and other primate herpesviruses will be continued.

Honors and Awards: None

Publications: None

Serial No. DBS 183
1. Laboratory of Viral Immunology
2. Section on Immunology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Investigations concerning viruses as possible etiologic agents in rheumatoid arthritis

Previous Serial Number: DBS 183

Principal Investigator: Robert M. Wilkes, M.D.

Other Investigators: Hope E. Hopps, M.S.
Barbara C. Meyer, M.A.
Harry M. Meyer, Jr., M.D.
Henry Roth, M.D.
John Decker, M.D.

Cooperating Units: Arthritis Clinic, D. C. General Hospital
Washington, D.C.
Arthritis and Rheumatism Branch, NIAMD, NIH

Man Years:

Total:	1.9
Professional:	1.6
Other:	0.3

Project Description:

Objectives: The major objective is to determine whether virus may play a role in the etiology of rheumatoid arthritis.

Major findings: During the past year, in a continuing search for infectious agents in rheumatoid arthritis, we have received 7 joint fluids from patients with rheumatoid arthritis and synovial tissue biopsies from 13 patients with rheumatoid arthritis, 2 normal patients (with trauma), and one lupus erythematosus patient. We have also received rheumatoid nodule biopsy tissue from 10 patients and cartilage from two patients. The fluids were inoculated on human embryonic kidney and WI-38 cells for virus isolation. Cells and supernatants were passaged up to two times. All specimens were tested for bacteria and mycoplasma and were negative.

Successful *in vitro* cultivation of cells was achieved in two of the rheumatoid fluids, 12 of the rheumatoid synovial tissues, two of the normal tissues, the lupas synovium, the two cartilages, and 6 of the rheumatoid nodules.

Previous efforts to isolate agents from such tissue cultures using standard techniques have not been successful. The heterokaryon, or cell fusion, technique has enjoyed modest success in rescuing known latent viruses. This technique was employed here. Synovial cells were fused with HeLa cells in the presence of betaprone inactivated Sendai virus. Heterokaryon formation was readily determined by examination of Giemsa stained preparations. The difference in morphology and tinctorial properties of the synovial and HeLa cells made it possible to distinguish and quantitate heterokaryon and synovial and HeLa homokaryon formation.

Heterokaryons were prepared from cell lines from 4 patients with rheumatoid arthritis, one rheumatoid nodule, one rheumatoid variant, and two with osteoarthritis. Heterokaryon preparations were observed for CPE for 10-14 days and then hemadsorbed. The supernatant fluids from such cultures were harvested at 10-14 days and examined for the presence of hemagglutinins. In addition supernatant fluids and sonicated cell free extracts were inoculated onto HeLa cells. These were observed for CPE for 2-3 weeks. The heterokaryons were also co-cultivated with HEK and WI-38 cells for 10-14 days, observed for CPE, and hemadsorbed. Slide preparations of 1 to 20 day old fused cells were examined for inclusion bodies using acridine orange and Giemsa stains. No viruses were isolated.

There has been much controversy about possible differences in viral susceptibility of rheumatoid and non-rheumatoid derived cell lines growing in culture. The viral susceptibility of 24 rheumatoid and non-rheumatoid cell lines was tested using ECHO-11, vesicular stomatitis virus, vaccinia, Herpes simplex, Newcastle disease virus, attenuated rubella virus HPV 77, and virulent rubella virus M33 BSC-1. No significant difference in susceptibility was found.

Significance to DBS Program and Bio-Medical Research: The possible association of a virus or other microbial agent in rheumatoid arthritis makes it essential for the Division to acquire competence in this area.

Proposed Course of the Project: The project will be continued with greater emphasis on studies of lupus materials.

Honors and Awards: None

Publications: None

Serial No. DBS 86
1. Laboratory of Viral Immunology
2. Section on Viral Biology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Detection and Characterization of Viruses

Previous Serial Number: DBS 86

Principal Investigator: John F. Thiel

Other Investigators: Lewellys F. Barker, M.D.
Ruth C. Dunlap, Ph.D.

Cooperating Units: Laboratory of Virology & Rickettsiology
Laboratory of Pathology

Man Years:

Total:	0.8
Professional:	0.8
Other:	-

Project Description:

Objectives: (1) To correlate physical and biological properties of viruses and (2) to develop more specific and more sensitive methods for detecting, identifying and classifying viruses.

Methods Employed: Cell culture techniques, electron microscopy, virus serology, fluorescence microscopy and virus infectivity titrations in tissue cultures.

Major Findings: Attempts were made to demonstrate the replication of HAA in chimp liver cells by blind passages of infected and control materials at weekly intervals. Materials were passed for twelve passages and tissue culture fluid from each passage of each material tested for replication of the HAA by radio immune assay technique. Positive inocula became negative after two passages indicating no replication. Cells from each passage were inoculated onto normal chimp liver cells in Petri dishes and grown for one week. The dishes were then fixed and examined for fluorescence by the indirect Coons reaction. No fluorescence was observed; similiar dishes were stained with acridine orange. Examination showed no changes in cell structure or morphology.

We were able to confirm the report of Purcell and Holland that it is possible to detect HAA in blood smears from HAA positive subjects. Using the indirect Coons reaction, fluorescence was observed in the matrix surrounding the blood cells, not in the cells themselves. Since it would be highly desirable to detect HAA in infected liver cells we are now preparing specific FITC conjugates from high-titre anti-HAA serum for use in the early detection of infection in such cells. This will involve the use of unfixed frozen cell sections.

Significance to DBS Program and Bio-Medical Research: The development and application of methods for detecting latent agents in cell cultures is directly related to the control of vaccine production in cell cultures.

Proposed Course of the Project: This project will be continued.

Honors and Awards: None

Publications: None

Serial No. DBS 321
1. Laboratory of Viral Immunology
2. Slow, Latent & Temporate Viruses Sec.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Hypersensitivity to viral vaccine antigens

Previous Number: None

Principal Investigator: J. A. Morris, Ph.D.

Other Investigators: C. W. Shaw, Jr.

Cooperating Units: None

Man Years:

Total:	0.4
Professional:	0.4 (Section established December 1971)
Other:	0

Project Description: Background: Most published information indicates that development of delayed hypersensitivity to myxovious vaccine components benefits the vaccine recipient, but information collected in several studies (Bull, D. R. and Burnet, F. M., Med. J. Aust. 1: 389, 1943; Fulginiti, V. A. et. al, Amer. J. Dis Child. 115: 671, 1968; Nader, P. R. et. al, J. Pediat. 72: 22, 1968) suggest that under certain conditions this immunologic reaction is harmful. In man inactivated measles and respiratory syncytial vaccines elicit, in some instances, harmful, and, in rare cases, lethal hypersensitivity reactions. In mice, influenza vaccine use is reported (Bull, D. R. and Burnet, F. M., ibid) to have been followed by more severe illness in vaccinated animals challanged with virulent influenza virus than in unvaccinated control mice. This finding reported more than 25 years ago, which suggests that the clinical manifestations of experimental influenza in mice might have an allergic basis, has not been followed-up in either animals or in man.

With this in view, studies were begun in January, 1972, (about 1 month after Section on SLT Viruses was established), to provide information on the behavior of mice following multiple exposures to influenza virus antigen. The experiments are in progress; the results will be described in subsequent reports.

Significance to DBS Program and Bio-Medical Research: If the possibility is real that some of the clinical manifestations of influenza infection might have an allergic basis, it is essential for the DBS to acquire information in this area.

Serial No. DBS 322
1. Laboratory of Viral Immunology
2. Slow, Latent & Temporate Viruse Sec.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July, 1, 1971 through June 30, 1972

Project Title: Study of latent agents in vaccine substrates.

Previous Number: None

Principal Investigator: J. A. Morris, Ph.D.

Other Investigators: C. W. Shaw, Jr.
Bernice Eddy, Ph.D.
Joseph Rogers

Cooperating Units: Experimental Virology Section, OD, DBS
Cell Culture Unit, LVR, DBS

Man Years:

Total: 0.4
Professional: 0.4 (Section established December, 1971)
Other: 0

Project Description: Background: Coggins (Proc. First Conference and Workshop on Embryonic and Fetal Antigens in Cancer, Oak Ridge, Tenn., May 24-26, 1971) reported that hamsters fetal cells possess an antigen cross-reactive in transplantation immunity tests with SV40 hamster tumor S antigen and further that normal pregnant hamsters develop antibody during the course of their pregnancy that react in complement fixation tests with the same S antigen. These observations suggest the possibility that certain antigens expressed in embryogenesis are similar to or identical with some of those present in SV40 transformed cells.

With this in mind cultured cells derived from the lung, kidney and skin-muscle of a 30-week fetus are being examined to determine whether the "Coggin" fetal antigen is detectably present. If the results of the current preliminary studies show that the "Coggin" antigen is present in human fetal cells, the current studies will be extended to examine cells of fetal origin now employed as vaccine substrates for presence of the same antigen.

Significance to DBS Program and Bio-Medical Research: Study of the biologic properties of cultured cells used as vaccine substrates is of immediate and long-range interest of the DBS and pertinent to its goals in the area of vaccine safety.

Serial No. DBS 323
1. Laboratory of Viral Immunology
2. Slow, Latent & Temporate Viruses Sec.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Studies concerned with slow, latent and temperate viruses.

Previous Number: None

Principal Investigator: J. A. Morris, Ph.D.

Other Investigators: C. W. Shaw, Jr.
C. G. Aulisio, Ph.D.
R. C. Reisinger, D.V.M.

Cooperating Units: Laboratory of Virology & Rickettsiology, DBS, NIH
Special Virus Cancer Program, NCI

Man Years:

Total:	0.2
Professional:	0.2
Other:	0

Project Description: Background: Severe injury has been observed as an immediate or early seguel of many immunization procedures. The incidence of such reactions is extremely low but when the do occur, especially when the brain is involved, the results may be devestating. The pathogenesis of these reactions is the subject of much speculation. The rarity of the reactions suggests that whatever the basic mechanism, it is dependent upon some individual peculiarity, one of which, it has been suggested, is a high blood level of endotoxin at the time of vaccination.

In the few months since the SLT Virus Section was established studies have gegun to develop an animal model to determine the role of circulating endotoxin on the animal's behavior following exposure to viral antigens. Results of preliminary tests suggest that Marek's disease in chickens will be a satisfactory model and studies are now being pursued in this model system, the findings of which will be described in subsequent reports.

Significance to DBS Program and Bio-Medical Research: Studies on the behavior of vaccine recipients following immunization procedures are an integral part of the mission of the DBS.

CONTRACT NARRATIVE
LABORATORY OF VIRAL IMMUNOLOGY

Fiscal Year 1972

Section on Immunology

HUNTINGDON RESEARCH CENTER (NIH-69-59)

Title: Preparation of Mycoplasma Antigens and Antisera

Contractor's Project Director: R. del Giudice

Project Officers (DBS): Hope E. Hopps, LVI
Michael F. Barile, LBP

Objectives: (1) To prepare standardized mycoplasma antigens and antisera which will be available for use by the Laboratory of Bacterial Products and other research groups within the DBS; (2) to provide a testing service for detection of mycoplasma in biological materials being used in the Division's various programs, e.g., tissue cultures, virus and rickettsial seed pools, sera, etc.; and (3) to provide services for isolation and identification of mycoplasmas from specimens obtained from other DBS contracts concerning adventitious agents; and (4) to survey coded samples of commercially obtained sera on order to assess current levels of serum contamination.

Methods Employed: Only purified, certified mycoplasma antigens are employed. Antisera are prepared in burros, donkeys or goats. Isolation studies are performed in broth and agar media. Identification is made on all isolates where possible. New test procedures are being studied to determine the best procedures for mycoplasma isolation.

Major Findings: Preparation of antigens, antisera and fluorescent antibody (FA) conjugates is proceeding on schedule. During the past 6 month period, 822 specimens were submitted to HRC for testing and identification (LVR-507, LVI-195 and LBP-120). There were 121 isolations made including M. orale, M. hominis, M. arginini, Acholeplasma laidlawii and Acholeplasma sp. Recently a program has been undertaken to study the current status of mycoplasma contamination in commercial lots of fetal borne serum. Prior to the screening of these sera, several methods for mycoplasma propagation are being tested in parallel in order to determine optimal growth conditions. When a system has been selected, coded samples will be sent by the Project Officer for testing.

Publications:

Tully, J., del Giudice, R. and Barile, M. Synony of M. arginini and M. leonis. International J. of Systematic Bacteriol. 32: 47-49, 1972.

del Giudice, R., Tully, J., Barile, M. Isolation and Characterization of M. conjunctival, sp.n. from sheep and goats with keratocon-juncturtis. J. Bacteriol. 5: 70-76, 1972.

Significance to DBS program and Bio-Medical Research: The mycoplasmas constitute an important group of organisms both with immediate regard to the regulatory control of vaccines and to their overall significance in human disease.

Proposed Course of Project: The project will continue.

Date Contract Initiated: September 28, 1966; renewed annually 1967-1971.

Present Level of Funding: $128,056

CONTRACT NARRATIVE
LABORATORY OF VIRAL IMMUNOLOGY

Fiscal Year 1972

Section on Immunology

YALE UNIVERSITY (PH-43-67-1463)

Title: A Statistical Approach to Human Karyotypy

Contractor's Project Director: Dr. Frank H. Ruddle

Project Officers (DBS): Hope E. Hopps, LVI
John C. Petricciani, LP

Objectives: The purposes of this contract are to determine the precise definition of the chromosome constitution of man and to seek the solution of certain problems relating to the chromosomal stability of cultured human cells.

Methods Employed: Leukocyte cultures were prepared from the cord blood of 4500 human newborns and approximately 1000 of their mothers. Slides of metaphase chromosomes were prepared and photographed. Idiograms were made from the photomicrographs and the chromosome arm length measurements were determined. High speed fully automated and semi-automated techniques of chromosome measurement have been utilized on a production basis. In addition, WI-38 cell cultures have been studied in detail in order to determine their karyotype at low, intermediate and high passage levels.

Major Findings: Karyologic studies on newborns have continued. There was a low frequency of structural abnormalities in newborn infants and the absence of even one infant with a high break frequency or more than one chromosome rearrangement. The mothers, however, shoed a twofold increase in breaks, threefold increase in fragments, and six to tenfold increase in rearrangements. More than 1% of the mothers (8/602) were found to have rearrangements. It seems reasonable to interpret the difference between the maternal and neonatal rates as reflecting in vivo differences, since the culture conditions were identical to both groups. There was no positive clinical correlation with high aberration rates except for therapeutic X-ray. Combining the neonatal and maternal samples five individuals in 2900 were observed with the same type of fragile area. The present study is the largest yet reported and this is the first report of frequencies of individuals with varying aberration rates. Because of the marked differences in the neonatal and maternal sample the results support the concept of the lymphocytes as a biological dosimeter for chromosomal aberration rate from birth onwards.

During this period WI-38 chromosome studies carried out by the orcein staining technique have been completed. We have also continued photographing of 10 or more cells from each of the newborns, the accumulation of additional data on chromosome break rates and their correlation with environmental factors. In addition, the previously developed quantitative technics were

adapted to include homolog identification by quinacrine mustard fluorescence and Giemsa 9 staining and these new technics were utilized for the more precise identification of many of the chromosomal variants found in the study population. These studies have demonstrated that a greatly enhanced reliability and resolution results when these quantitative technics are coupled with homolog identification. Additional epidemiological studies were also carried out.

Significance to DBS Program and Bio-Medical Research: This contract has two primary objectives. The first objective is to establish a reliable definition of the chromosome constitution of man and the second is the solution of problems relating to stability of continuous diploid human cell lines *in vitro* and the karyotypic response of these cell lines to infection with selected adventitious agents.

A precise definition of the chromosome constitution of man and a better understanding of the events associated with senescence in cultured human cells will allow the Division to make recommendations concerning the use of cultured human cells in vaccine production and may lead to new tissue culture techniques for evaluating the safety of biologics.

Proposed Course of Project: Work under this contract was initiated June 29, 1967. The contract will terminate on June 29, 1972.

Current Annual Level: $71,150

CONTRACT NARRATIVE
LABORATORY OF VIRAL IMMUNOLOGY

Fiscal Year 1972

Section on Immunology

GULF SOUTH RESEARCH INSTITUTE (NIH-70-2299)

Title: Development of Rabbit Diploid Cell Strains Suitable for Use in Production of Human Vaccines

Contractor's Project Director: Dr. Benjamin Sweet

Project Officers (DBS): Hope E. Hopps, LVI
John C. Petricciani, LP
Douglas Lorenz, LP

Objectives: The purpose of this contract is to develop diploid cell strains from fetal tissues of the New Zealand white rabbit (Oryctologus cuniculi) which would be suitable for use in the production of viral vaccines (Human).

Methods Employed: Fetal tissues from selected rabbits are propagated in vitro under optimal growth conditions. Cell strains are characterized with respect to growth potential, viral susceptibility, chromosomal consitution and freedom from adventitious agents.

Major Findings: A total of 19 different rabbit cell lines supplied by Delta Primate Research Center (Tulane University) were cytogenetically screened by the principal investigators. (An additional 4 lines are in progress.) This included, wherever possible, cell morphology, polyploid indices, sex chromatin and chromosome counts. Furthermore, detailed karyotypic analysis of heteroploid and diploid cells from 18 different lines were completed. The passage level at which examinations were made ranged from the 10th to 54th. These lines represented the following:

a. Those derived from pooled tissue of fetuses from 6 New Zealand rabbits including kidney (7) and lung (4). All were fibroblast type cultures.

b. Heart (2), skin (2), lung (1), and kidney (3) cultures derived from individual fetuses from two different dams. The lines were either fibroblastic or epithelial in character.

An analysis by one senior investigator (N. Bianchi) using his techniques (coverslip method) indicated that of the 11 cultures derived from pooled fetal tissue, 8 lines showed polyploidy or heteroploidy of more than 25% in the cells analyzed. The remaining 3 lines which were analyzed at passage 17019 had a polyploid index ranging between 3 and 20% and 10-12% of cells analyzed were heteroploid. These latter lines are presently considered questionable because of the presence of one apparently consistent

abnormal chromosome. In hyperdiploid and hypodiploid cells the chromosome gain or loss appeared to be selective for certain chromosomes or chromosome groups. In hyperdiploid cells, one medium size acrocentric (telocentric) was the most common and approximately 69% of such cells showed this abnormality. These findings may be inherent in the rabbit species itself, and the hypothesis is under test. This abnormality was also found in cell lines derived from individual fetuses.

Analyses of cell strains derived from individual rabbit fetuses were also carried out by both senior investigators. Of 8 such lines, 4 demonstrated polyploidy or heteroploidy of greater than 25%. The remaining 4 represented the following:

a. A skin fibroblast culture at passage 12, which has 8% heteroploidy and polyploidy.

b. A heart epithelial culture at passage 11, which has 8% polyploidy and 17% heteroploidy.

c. A lung fibroblast culture at passage 34 with 3% polyploidy and 7% heteroploidy. Approximately 7% of the diploid set are quasidiploid.

d. A kidney epithelial line which at passage 10 had a polyploid index of 6% with 4% heteroploid cells and at passage 33 exhibited 23% polyploidy and heteroploidy. At the latter passage, approximately 22% of the diploid set were considered quasidiploid.

Studies have been initiated on primary cultures from adult and 28 day old fetal tissue. These studies will give us a beginning baseline for the "normal" chromosomal as well as the heterochromatin patterns in primary tissue. The results obtained can then be compared to the patterns observed in cell strains derived from these tissues, and aid in selecting those cell lines which should be followed further.

Significance to DBS Program and Bio-Medical Research: The availability of diploid cell strains for vaccine use is another important approach to obtaining safe vaccines.

Proposed Course of Project: During the coming year, candidate strains will be selected and further characterized as regards karyology, virus susceptibility, growth patterns, tumorigenicity and freedom from adventitious agents.

Date Contract Initiated: June 24, 1970; renewed 1971 and 1972.

Current Annual Level: $225,574

CONTRACT NARRATIVE
LABORATORY OF VIRAL IMMUNOLOGY

Fiscal Year 1972

Section on Immunology

TULANE UNIVERSITY (NIH-70-2300)

Title: Development of Rabbit Diploid Cell Strains Suitable for Use in Production of Human Vaccines

Contractor's Project Director: Dr. Ambhang D. Felsenfeld

Project Officers (DBS: Hope E. Hopps, LVI
John C. Petricciani, LP
Douglas Lorenz, LP

Objectives: The purpose of this contract is to develop diploid cell strains from fetal tissues of the New Zealand white rabbit (Oryctologus cuniculi) which would be suitable for use in the production of viral vaccines (human).

Methods Employed: Fetal tissues from selected rabbits are propagated in vitro under optimal growth conditions. Cell strains are characterized with respect to growth potential, viral susceptibility, chromosomal constitution and freedom from adventitious agents.

Major Findings: Six growth media have been employed during the period reported. Tissues from 13 fetus from 2 dams were processed individually. Sixty-one cell lines were established. In addition, 31 cell lines established during previous work are being carried, a total of 92 cell lines. Frozen stock is prepared in all lines. Mostly fibroblast-type cells developed. Some cell lines from lung and kidney are epithelium-like, but few consist of true epithelium cells. The evaluation of the reference rubella strain is in progress. The asepsis employed in the laboratory appears to be effective because the contamination rate is less than 1% and is mostly due to bottle sterilization problems. Contamination with yeasts has not been observed. Growth termination of a few cell lines has been observed after the 10th passage, heralded by degenerative changes. Microbiological and histological examination of the dams did not yield pathogenic microorganisms nor findings of histopathological nature. Most cell lines derived from rabbits have a tendency to lose their diploid characteristics in late passages. Therefore our principal task is to select such cell lines that do not show this trend and, at the same time, support the growth of the rubella virus. Three of the cell lines developed could possibly be considered as candidates for viral vaccine productions.

Significance to DBS Program and Bio-Medical Research: The availability of diploid cell strains for vaccine use is another important approach to obtaining safe vaccines.

Proposed Course of Project: This portion of the Rabbit Diploid Cell Program is complete and the contract will terminate on June 24, 1972. Cell lines which have been developed at the Tulane Laboratories will be transferred to the Gulf South Research Institute for further study.

Date Contract Initiated: June 24, 1971

Current Annual Level: $157,598

CONTRACT NARRATIVE
LABORATORY OF VIRAL IMMUNOLOGY

Fiscal Year 1972

Section on Immunology

WYETH LABORATORIES (NIH-70-2298)

Title: Development of Rabbit Diploid Cell Strains Suitable for Use in Production of Human Vaccines

Contractor's Project Director: Dr. Howard Tint

Project Officers (DBS): Hope E. Hopps, LVI
John C. Petricciani, LP
Douglas Lorenz, LP

Objectives: The purpose of this contract is to develop diploid cell strains from fetal tissues of the New Zealand rabbit (Oryctolagus cuniculi) which would be suitable for use in the production of viral vaccines (human).

Methods Employed: Fetal tissues from selected rabbits are propagated in vitro under optimal growth conditions. Cell strains are characterized with respect to growth potential, virus suceptibility, chromosomal constitution and freedom from adventitious agents.

Major Findings: Seven fetal rabbit cell strains derived from inbred Strain III rabbit #760 were cultivated in vitro to determine their finite growth potential, their cytogenetic complement at various cell passage levels, and their ability to support the growth of rubella virus. The seven cell strains, two skin, three lung and two cornea, have now been adapted to their in vitro environment. Three rabbit strains, Skin 16, Cornea 15 and Lung 20 showed a deviation from normal diploid karyology at the last tissue culture passage tested. One strain, Lung 16, has remained diploid throughout 74 tissue culture passages. Six of the seven rabbit strains were found to support the growth of rubella virus. Lung cultures appeared best as they produced from 4 to 5 logs of virus. Virus multiplication in all cell strains was demonstrable only by interference. Sixteen rabbit cell strains derived from lung, skin and corneal tissue have been initiated from inbred Strain III rabbit #623. All are approximately at their 40th in vitro cell passage. Representative strains are now being studied cytogenetically and assayed for their rubella virus sensitivity. Primary rabbit tissues were also studied karyologically with variations in polyploidy observed depending on the tissue used. Similar studies with primary tissues from inbred Strain III rabbits are in progress.

Significance to DBS Program and Bio-Medical Research: The availability of diploid cell strains for vaccine use is another important approach to obtaining safe vaccines.

Proposed Course of Project: The program of this laboratory will now be directed toward the completion of karyologic studies on primary cultures from both inbred and wild New Zealand rabbit tissues and to the detailed characterization of several candidate lines developed during the past 2 years. These lines will be examined with regard to karyology, virus susceptibility, growth patterns, tumorigenicity and freedom from adventitious agents.

Date Contract Initiated: June 23, 1970; renewed 1971 and 1972.

Current Annual Level: $238,490

CONTRACT NARRATIVE
LABORATORY OF VIRAL IMMUNOLOGY

Fiscal Year 1972

STATE OF NEW JERSEY DEPARTMENT OF HEALTH (NIH-DBS-70-2026)

Title: Epidemiologic Study of Transfusion-Associated Hepatitis

Contractor's Project Director: Martin Goldfield, M.D.

Project Officers (DBS): Lewellys F. Barker, M.D., LVI
Robert J. Gerety, M.D., LVI
Joseph A. Markenson, M.D., LVI

Objectives: This contract is to study the significance of positive tests for hepatitis B antigen (HB Ag) in blood donors in New Jersey, particularly as related to the sensitivity and specificity of tests in detecting those units of blood associated with hepatitis in recipients, and to accumulate and study a bank of blood donor specimens on which the consequences of transfusion to recipients are documented.

Methods Employed: Agar gel diffusion, complement fixation, counter-electrophoresis and radioimmunoassay techniques have been used to test sera for HB Ag in sera collected from blood donors and recipients in New Jersey.

Major Findings: Tests for HB Ag have been performed by a variety of methods on several hundred thousand donor bloods collected in New Jersey. The relative sensitivity of methods may be ranked: AGD, CEP, CF, RIA in order of increasing sensitivity. Information derived from recipients followed prospectively during the first year of the contract (1969) and by analysing all reported cases and cases recorded in hospital record rooms throughout the duration of the contract, reveals that removal of bloods found positive by current testing methods will reduce the risk of post transfusion viral hepatitis, type B, but not eliminate the risk. Evidence has been acquired indicating that between 30 and 40% of post-transfusion hepatitis in New Jersey may be caused by hepatitis A virus or other agents. Correcting for this portion of post-transfusion hepatitis, which could not conceivably be affected by screening donors for HB Ag, the expected reduction in total recognized cases of hepatitis that may be accomplished by screening with the most sensitive currently available method is between 40 and 50%. The most sensitive method currently available is the solid phase RIA developed by Abott Laboratories. Extensive evaluation of this method has been made in New Jersey during the past year. In addition, studies of antigenic subtypes and their significance are being conducted and attempts to evaluate and improve antigen detection methods are continuing.

Significance to DBS Program and Bio-Medical Research: The evaluation and improvement of methods for detecting HB Ag in blood donors is directly

related to research and regulatory DBS programs directed towards the safety of blood and blood products. Extensive epidemiologic information about viral hepatitis, type B, is an essential foundation to achieving control of this disease by immunization and other public health measures.

Proposed Course of Project: This project will be continued.

Date Contract Initiated: October 1, 1969

Current Annual Level: $226,800.00

CONTRACT NARRATIVE
LABORATORY OF VIRAL IMMUNOLOGY
Fiscal Year 1972

HAZLETON LABORATORIES, INC. (NIH-71-2040)

Title: Housing and Special Care of Chimpanzees

Contractor's Project Director: Dan W. Dalgard, D.V.M.

Project Officers (DBS): Lewellys F. Barker, M.D., LVI
Francis V. Chisari, M.D., LP

Objectives: This contract is to provide facilities and technical support for a program designed to evaluate chimpanzees as an animal model for studies of viral hepatitis.

Methods Employed: Animals are pre-screened for HB Ag and anti-HB Ag prior to purchase; antigen and antibody free animals are selected for inoculation studies. Serial enzyme determinations and liver biopsies are obtained prior to and post-inoculation.

Major Findings: To date six animals have been inoculated with HB Ag positive materials and evidence of infection has been obtained in five of these six. Infection of three of these five represents a second passage of an animal initially infected with the NIH plasma pool. Four more animals are ready for inoculation and a fifth chronically infected HB Ag carrier animal is being held as a source of antigen and infected tissue. Further details of these studies may be found under project report No. DBS-137.

Significance to DBS Program and Bio-Medical Research: The development of animal models for viral hepatitis, type B, is essential for studying active and passive immunization as well as the pathogenesis and carrier state of this disease.

Proposed Course of Project: This project will be continued.

Date Contract Initiated: November 6, 1970

Current Annual Level: $23,100.00

CONTRACT NARRATIVE
LABORATORY OF VIRAL IMMUNOLOGY

Fiscal Year 1972

HAZLETON LABORATORIES, INC. (NIH-DBS-72-2050)

Title: Investigate Susceptibility of Sub-Human Primates to Human Hepatitis

Contractor's Project Director: Dan W. Dalgard, D.V.M.

Project Officers (DBS): Lewellys F. Barker, M.D., LVI
Douglas E. Lorenz, Ph.D., LP

Objectives: This contract is to provide facilities and technical support for a program designed to evaluate a variety of monkey species as animal models for studies of viral hepatitis.

Methods Employed: Pre-screened animals, free of HB Ag and anti-HB Ag, are inoculated with a variety of infectious materials and followed with serial enzyme determinations, liver biopsies and serologic studies.

Major Findings: Three species of South American monkeys (Squirrel monkeys, Owl monkeys and Capuchin monkeys) have been obtained. To date only the Squirrel monkeys have been inoculated and it is too early to judge whether or not they will be susceptible to viral hepatitis, Type B. Baseline studies prior to inoculation are being obtained on the other two species.

Significance to DBS Program and Bio-Medical Research: The development of animal models for viral hepatitis, type B, is essential for studying active and passive immunization as well as the pathogenesis and carrier state of this disease.

Proposed Course of Project: This project will be continued.

Date Contract Initiated: December 1, 1971

Current Annual Level: $76,500.00

CONTRACT NARRATIVE
LABORATORY OF VIRAL IMMUNOLOGY

Fiscal Year 1972

UNIVERSITY OF NORTH CAROLINA, SCHOOL OF MEDICINE (NIH-69-2197)

Title: To Investigate the Application of Large Volume Zonal Centrifugation, Purification, Analysis, and Testing of Biological Material

Contractor's Project Director: D. Gordon Sharp, Ph.D.

Project Officers (DBS): Lewellys F. Barker, M.D., LVI
Ruth C. Dunlap, Ph.D., LVI

Objectives: To develop efficient methods for concentrating and partially purifying the hepatitis associated antigen.

Methods Employed: Centrifugation in the BXIV zonal rotor, pressure dialysis and concentration, electron microscopy and standard serologic methods for quantitating hepatitis B antigen (HB Ag).

Major Findings: Large volume zonal centrifugation of human sera has produced pruified concentrates of HB Ag for use in passive hemagglutination, radioimmunoassay, antigenic analysis, animal immunization and experimental infection studies. Fractions isolated in one isopynic sucrose run made on chimpanzee serum transmitted viral hepatitis, type B, to another animal, in contrast with antigen positive fractions from a sucrose rate zonal run which did not transmit hepatitis to another chimpanzee.

Progress has been made towards quantitative electron microscopy of the HB Ag particles by differential centrifugation and negative staining techniques.

Significance to DBS Program and Bio-Medical Research: Preparation of purified HB Ag concentrates has seen essential to the seriologic and biologic studies of viral hepatitis, type B which are directly related to the program of the Division.

Proposed Course of Project: This project will be discontinued as adequate supplies of purified materials for present needs have been prepared.

Date Contract Initiated: July 1, 1969

Current Annual Level: $38,809.00

CONTRACT NARRATIVE
LABORATORY OF VIRAL IMMUNOLOGY
Fiscal Year 1972

Section on Immunology

YALE UNIVERSITY (PH-43-67-1463)

Title: A Statistical Approach to Human Karyotypy

Contractor's Project Director: Dr. Frank H. Ruddle

Project Officers (DBS): Hope E. Hopps, LVI
John C. Petricciani, LP

Objectives: The overall objectives of this continuing contract have been the precise definition of the chromosome constitution of man as well as a detailed analysis of the chromosomal patterns of the WI-38 cell line during the major phases of its life span.

Methods: During the current year, new banding techniques have been applied to a study of WI-38 cells and to specimens obtained from 40-50 families in the New Haven Newborn Study.

Major Findings: Populations of WI-38 cells were examined at passages 20, 27-40 and > 41, i.e., early, middle and late stage. A total of 250 cells were sampled: 21 from early phase, 98 from middle phase and 131 from late phase. Observations revealed no detectable ulteration in the banding pattern of chromosomes during finite life (FL), indicating that the chromosomes appear to be unaltered during the so-called aging process. Results of banding studies of the banding patterns in families participating in the New Haven Newborn Study are being analyzed.

Significance to DBS Program and Bio-Medical Research: A precise definition of the chromosome constitution of man and a better understanding of the events associated with senescence in cultured human cells will permit the Division to make recommendations concerning the use of cultured human cells in vaccine production and may lead to new tissue culture techniques for evaluating the safety of biologics.

Proposed Course of Project: Work under this contract was initiated June 29, 1967. The contract will be terminated this year.

Current Annual Level: $71,150

Serial No. _____
1. Experimental Virology
2. Office of the Director
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Experimental Virology

Previous Serial Number: Same

Principal Investigator: Bernice E. Eddy, Ph.D.

Other Investigators: None

Cooperating Units: None at a cost to the government

Man Years:
Total: 3
Professional: 1
Other: 2

Project Description:

To investigate the safety of biological products, especially in relation to the oncogenicity of viruses and means of preventing neoplastic development; and to investigate new viruses and other agents in relation to their importance in human disease and for exploring the possibility of preventing diseases that at present are uncontrolled.

Methods Employed: Conventional methods of propagating viruses in cell cultures and in animals, and immunological methods for the detection of viral- or cellular-induced antigens or antibodies.

Major Findings: A continuing investigation in the section relates to the question of viral oncogenesis, and to the circumstances under which neoplastic changes in cells can be enhanced or suppressed by an oncogenic virus or by antibodies induced by the virus or neoplastic cells.

Methods for recovering simian virus 40 from transplanted hamster tumors have been and are continuing to be employed in attempts to recover virus from human carcinomas. To date, two viruses have been recovered. The role of these viruses, if any, in the induction or maintenance of carcinomas are, as yet, unknown. Studies are underway to determine if the viruses are able to transform "normal" cell cultures or to induce tumors in

hamsters. In collaboration with an investigator at George Washington University and another investigator in Ottawa efforts are being made to find out if these viruses react immunologically with sera from patients with similar carcinomas.

A study of the effect of living mycobacterium bovis strain BCG, (BCG) and more recently, vaccinia, on virus-induced tumors in hamsters is underway. BCG has, under certain conditions, a deterring effect on tumors induced by adenovirus type 12, but has done little or nothing to hinder tumors induced by simian virus 40 (SV40). Conditions are being varied in an attempt to demonstrate an inhibiting effect on SV40-induced tumors.

The effect or lack of effect on transformation by different nononcogenic viruses is being tested in SV40-infected cell cultures.

Use is being made of an adenovirus type 12 strain which has been rendered non-oncogenic or almost nononcogenic to find out if any viral or bacterial vaccines will activate the induction of tumors in hamsters.

Efforts are being continued to find a method for recovering infectious adenovirus type 12 from adenovirus type 12-induced hamster tumor cells. Like many tumors in man, infectious virus cannot be recovered from these tumors.

Significance to Biomedical Research and the Program of the Division: This program is of major significance in developing an understanding of hazards that may exist in proposed biological products and in providing information on the importance of the relationship of immunity and oncogenesis.

Proposed Course of Project: Work will be continued on immunologic or other effects of various viruses in relation to oncogenicity, employing techniques and experimental protocols similar to those above but varied in different parameters.

Honors and Awards:

- On Advisory Board, American Type Culture Collection.
- Chairman, Medical Science Panel, Washington Academy Science.
- Member, Editorial Board of Diplomate Forum, The American Board of Medical Microbiology.

- Member of Nominating Committee, Washington Branch of American Society of Microbiology

- Asked to speak at the Association Internationale de Standardisation Biologique at Annecy, France, Sept. 20-24, 1972.

Publications:

1. Eddy, B. E.: Viral infectivity or oncogenesis due to viruses in hamsters. In Hamster Pathology. Hamburger, F. (Ed.), S. Karger Publishing Company, Basel/New York, pp. 455-497, 1972.

2. Eddy, B. E.: Problems in the detection of the oncogenic potential of viruses or cell cultures. Progress in Immunobiological Standardization. S. Karger Publishing Company, Basel/New York. In Press.

Annual Report of the Biometrics Section
Division of Biologics Standards
July 1, 1971 through June 30, 1972

I. MISSION

The primary responsibility of the Biometrics Section, Division of Biologics Standards, is to insure that efficient statistical techniques are available and used in the scientific operations of the Division. For the most part, adequate techniques are available in the literature, but on occasion this will not be so. Even where appropriate techniques have been discussed, the literature may be scattered, highly technical, and contradictory, requiring study and comparison for adequate exploitation.

II. ANALYSES SUMMARY

In Fiscal Year 1972, 77 specific tasks were initiated in the Section. Of these, 66 were completed, 2 were cancelled or suspended, and 9 are continuing. A listing of all tasks in the Section is given in the addendum.

III. COMPUTATION DEVELOPMENTS

1. A time-sharing terminal was installed in the Section on February 4, 1969, permitting an ultimate intimate incorporation of the computer into the statistical processing activities of the Section. The advantages of computer processing are limited by two considerations: (1) the extent of calculations vis a vis data input and output and (2) the availability of suitable software in the form of previously prepared programs which may be utilized with a minimum of adaptations to each specific calculation. Statistical treatment of experimental data involves a very favorable ratio of computation to input-output, but places a very heavy strain on the availability of appropriate programs. Consequently a fund of such programs must be built up over time and very sophisticated program development employed in order that the great variety of data formats can be accommodated with a minimum of program adaptations at the time of execution. Limited assistance has been obtained from commercially supplied programs; in particular, programs for inverting matrices, solving simultaneous linear regressions, polynomial equations, matrix manipulations, chi-square, and the like.

2. Principal computer program developments during this report period are: (1) a three-way analysis of variance expected value program, (2) a program for calculating RX2 analyses of variance with disproportionate subclass numbers, (3) addition of significance level calculations to existing Bartlett's test and single degrees of freedom programs, (4) a generalization of the existing covariance program to permit great flexibility in its use, (5) some simplification of the existing DISSEV program, (6) the development of a new program for calculating

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probabilities where not all treated subjects are susceptibles, (7) a series of programs for table preparation, (8) a program to compute an observed frequency distribution, (9) an extensive revision of the existing parabolic regression program to improve output, (10) adaptation of the elaborate bioassay program under development at DCRT over the past six to eight years, so that input can be done at our remote terminal, (11) a relative potency and/or unitage program, (12) a general sort program, (13) a program for fitting normal probability programs, and (14) a program for employing all the information in bioassay experiments with three possible outcome classes, so called Trichotomous assay.

3. Absence of the one trained computer program specialist has resulted in only slight pursuit of the alternatives to the General Electric Mark II BASIC programming system which is the chief reliance of the calculational activities of the Section. A written request for approval in principle of placing a contract for such a survey was initiated 7 April 1972 after a fruitless period of administrative discussion.

IV. METHODOLOGICAL RESEARCH

In all of the items described below, the data were obtained by DBS scientists or were submitted by manufacturers. The language here is biological, but in every case what is being described is that the questions at issue were treated statistically and the conclusions stated meet the conventional probability tests of correctness.

1. Serological comparisons demonstrated that Rhesus monkeys are serologically closer to man than are cercopithecus monkeys, though the two monkey species are closer to each other than either is to man.

2. A proposed chromosome screening test for WI-38 cells was examined for adequacy of the statistical treatment. It proved very deficient and was subsequently withdrawn.

3. A Pseudomonas Vaccine was tested in a trial and asserted to be clearly established as effective on the basis of the data obtained. Statistical analysis showed that the conclusion was not yet established. Further testing was recommended.

4. Trials of Poliovirus Reference stability were conducted by a manufacturer and by DBS. The standard proved stable and well-behaved in DBS hands.

5. A manufacturer's IND study design was reviewed. While apparently sound, it did not seem to promise the superiority the manufacturer claimed. Further study is recommended.

6. In some clinical trials relatively few of the population are susceptibles, perhaps 5-15%, but a way of discriminating between them

is possible. Hence of treated patients 85-95% will be "successes" irrespective of the efficacy of the treatment. The effect of this is to greatly increase the numbers of patients required for an adequate trial, how much is a matter of calculation. A computer routine was developed to obtain answers for a particular design. It can be used to provide quick answers under changed conditions.

7. An earlier study established Antigen E as the principal factor in weed hay fever. The data accumulated in this study were enough to show that the situation is essentially similar in grass hay fever in terms of GpI content.

8. Measurement of plaque size is of course easy and convenient and, if differences in mean plaque size or plaque size variability could be shown to be species or strain specific could be used as markers. However, plaque size varies widely even on a single plate. Data from several strain-selection combinations were examined and some encouragement developed that these statistics could serve as markers, particularly under refined conditions.

9. A small set of data on influenza potency tests were examined by the probit program under development by collaboration with DCRT for the past eight years or so. The data were not well adapted to calculation by probits and required an assumption to yield results which probably would not have been necessary had the program encountered these data under less hurried circumstances.

V. OPERATIONAL PROBLEMS

Ability to furnish Division investigators with prompt, adequate, and accurate statistical analyses degenerated during the first half of the report period but improved somewhat during the last half. Reluctance of the Division management to support efforts to rectify the conditions persists.

VI. The research activities of the Section grow out of its consulting and statistical processing activities, which cannot be anticipated. While some slight increase in the computational facilities of the Section would prove extremely helpful, in particular a more modern higher-capacity terminal, the primary need is to increase exploitation of existing capabilities. It has not proved possible (a) to gain general acceptance of the computer as the central tool in the work of the Section, (b) to add staff competent and adapted to remote console computer operation, or (c) to gain permission to place a contract to improve conditions.

Serial No. DBS 111
1. Office of the Director
2. Biometrics Section, DBS
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1971 through June 30, 1972

Project Title: Development of Statistical Methodology Applicable to Biologics Control

Previous Serial Number: DBS 111

Principal Investigator: Clifford J. Maloney, Ph.D.

Other Investigators: Harold Baer, Ph.D., Charles Manclark, Ph.D., Thomas C. O'Brien, Ph.D., Dr. Edward Seligmann, Mrs. Elizabeth B. Paul, Mr. Fred S. Yamada

Cooperating Units: Laboratory of Bacterial Products, Laboratory of Virology and Rickettsiology, Laboratory of Blood and Blood Products, Math/Stat Programming Section, DCRT

Man Years:

Total:	0.6
Professional:	0.4
Other:	0.2

Project Description:

Objective: As need arises from the research activity of the Division for statistical technique not adequately treated in the literature and when it appears that a need for such technique will continue on an appreciable scale in the future, theoretical and computational work is undertaken to develop appropriate technical tools.

Subproject 1: Evaluation of the efficacy of a treatment in a population where 90% are immune was required.

Subproject 2: Evaluation of the true source of efficacy of grass pollens was studied in a very few patients.

Subproject 3: A need for an objective discrimination between two pyrogen tests arose.

Subproject 4: Conformity to conditions of validity in a test for potency of influenza vaccine developed.

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Subproject 5: Pertussis testing in mice has for years involved recording of effects less severe than death. Use of this information in routine testing has been obtainable in the literature for over a dozen years, but requires computer processing.

Methods:

Subproject 1: Calculation of the effects of treatment where some patients are immune involves laborious but straightforward probability calculations. These are easily accomplished by joint use of tables and a computer. Where the table is inadequate, a method was devised to perform all calculations by computer.

Subproject 2: Despite the roughness of the data, conventional statistical methods were adequate for this problem.

Subproject 3: The problem here, while of long standing, is one where recognized statistical procedures are not known.

Subproject 4: The statistical problem raised by this problem calls for a choice between various transformation of data. In the past, the choice has tended to be made on a crude basis. Some recent papers bear on this problem.

Subproject 5: The statistical procedures appropriate to the treatment of this problem are of two sorts. The first are standard bioassay techniques which have been in the process of adaptation to the DCRT computer over the past eight years. The second involves the programming of a procedure available for over 12 years but never before programmed for application by computer.

Findings:

Subproject 1: A computer program was devised adequately to solve the immediate presented problem. The program remains available for any future demand.

Subproject 2: Statistical analysis of the limited data available permitted drawing deductions of a degree of detail and firmness much beyond those which eye appraisal would support, and permitted a degree of agreement not usually forthcoming when judgments are based on subjective appraisal.

Subproject 3: Ad hoc combined computation-data-analysis permits a clear-cut discrimination and suggestions for improving the chosen test.

Subproject 4: This problem may be unique in the history of vaccine regulation in the United States in the diversity of conclusions accepted in authoritative quarters. That the data were not submitted for treatment in time to remove the inadequacies of the current computer program is therefore particularly to be regretted.

Subproject 5: Subject matter findings await completion of the two computer programs involved. Both would have been completed long ago had adequate technical support been forthcoming before the development of a crisis.

Significance to Bio-Medical Research and the Program of the Division:

The sudden recognition that formal statistical methods contribute to drawing sound conclusions from the results of experiments and tests, protect against incorrect deductions, and contribute to an increase in efficiency in experimentation and in regulation is probably the most important result--if it occurs. The development of the program for Trichotomous bioassay should be widely useful.

Honors and Awards: None

Maloney, C.J.: What is probability? Proceedings, 38th International Statistical Institute, August 10-20, 1971.

Maloney, C.J.: Multiple comparisons revisited. Proceedings, Biometric Society Meetings (ENAR-WNAR), Fort Collins, Colorado, August 23-26, 1971.

Maloney, C.J.: Combining multiple attribute outcomes into an overall index. Proceedings, IASPS Symposium on Pollution, Harvard University, August 30-September 3, 1971.

Maloney, C.J.: Disease severity index. (Revised). Proceedings, 17th Army Design Conference, October 1971.

Maloney, C.J., Yamada, F.S.: A computer program for trichotomous bioassay. Proceedings, 17th Army Design Conference, October 1971.

Maloney, C.J.: The limits of measurement. Proceedings, 10th Colloquium on Atmosphere Pollution, Paris, France, May 3-5, 1972.

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TABLE I

SUMMARY OF STATISTICAL ANALYSES FOR FISCAL YEAR 1972

SAN	DATE INITIATED	DATE COMPLETED	TITLE	RESEARCHER
327	April 1971	May 1971	LD50 Titration I	Dr. Manclark
328	May 1971	May 1971	Geometric Means	Dr. Baer
329	May 1971	May 1971	HSD50 Determinations II	Dr. Manclark
330	May 1971	May 1971	Geometric Means	Dr. Friedlaender
331	May 1971	May 1971	Kingspoint I and II	Dr. O'Brien
332	May 1971	July 1971	Titration Curves	Dr. O'Brien
333	May 1971		Collaborative Assay Study	Dr. Seligmann
334	May 1971	June 1971	ED50 Calculations	Dr. Hardegree
335	May 1971		Collaborative Assay Study	Dr. Seligmann
336	May 1971	June 1971	Kingspoint I- HI Sera	Dr. O'Brien
337	June 1971	June 1971	BCG Lymphocyte	Dr. Chaparas
338	June 1971	June 1971	Outlier Significance	Dr. Geller
339	June 1971	July 1971	Cercopithecus and Rhesus Sera	Dr. Rowe
340	June 1971	June 1971	Mortality Rates - 10 mice	Dr. Singer
341	June 1971	June 1971	HSD50 Determinations III	Dr. Manclark

TABLE I - continued

SAN	DATE INITIATED	DATE COMPLETED	TITLE	RESEARCHER
342	June 1971	June 1971	Chi-Square Tests	Dr. O'Brien
343	June 1971	September 1971	Chromosome Screening Test	Dr. Petricciani
344	June 1971	July 1971	Pseudomonas Vaccine	Dr. Feeley
345	June 1971	July 1971	HSD50 Determinations IV	Dr. Manclark
346	July 1971	July 1971	Standard Deviations	Dr. Barker
347	July 1971	cancelled	Standard Variability	Dr. Hardegree
348	July 1971	July 1971	Geometric Means	Dr. Friedlaender
349	July 1971	August 1971	Poliovirus Reference Stability (II)	Dr. Parkman
350	July 1971		Human PPD Trials	Dr. O'Malley
351	July 1971	July 1971	Follow Up Sampling	Dr. Gibson
352	July 1971	July 1971	IRS Comparisons	Dr. O'Brien
353	July 1971	July 1971	Kingspoint II - Vaccinia HI	Dr. O'Brien
354	July 1971	July 1971	Geometric Means	Dr. Baer
355	July 1971	July 1971	Chi-Square Tests	Dr. O'Brien
356	July 1971	September 1971	Geometric Means	Dr. Friedlaender

TABLE I - continued

SAN	DATE INITIATED	DATE COMPLETED	TITLE	RESEARCHER
357	July 1971	October 1971	ATG Study	Dr. O'Malley
358	July 1971	August 1971	Tetanus Toxoid IV	Dr. Hardegree
359	August 1971	August 1971	Respiration Protracta	Dr. Singer
360	August 1971	December 1971	Toxoid Variability	Dr. Hardegree
361	August 1971	December 1971	Response Dose Sensitivity	Dr. Hardegree
362	August 1971		Coral Snake Venom	Dr. Seligmann
363	August 1971	November 1971	Aberration Specificity	Dr. Petricciani
364	August 1971	September 1971	CCA Values	Dr. O'Brien
365	August 1971	May 1972	Pyrogenic Effect	Dr. Hochstein
366	August 1971	September 1971	Uncorrected CCA Values	Dr. O'Brien
367	September 1971	May 1972	Tetanus Toxoid V	Dr. Hardegree
368	September 1971	January 1972	Correlation of Antibodies and Cells	Dr. Petricciani
369	September 1971	December 1971	ED50 Calculations II	Dr. Hardegree
370	September 1971	November 1971	Anti Rh Factor	Mrs. Paul
371	September 1971	November 1971	Chemical Groups	Dr. Friedlaender

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TABLE I - continued

SAN	DATE INITIATED	DATE COMPLETED	TITLE	RESEARCHER
372	September 1971	cancelled	Allergenic Extracts	Dr. Baer
373	September 1971	September 1971	Chi-Square Test on Polio Vaccine	Dr. Morris
374	September 1971	November 1971	One-failure probability	Mrs. Paul
375	October 1971	November 1971	Differences between A and G Viruses	Dr. Albrecht
376	October 1971	October 1971	Geometric Means	Dr. Baer
377	October 1971	October 1971	Random Numbers	Dr. Chaparas
378	October 1971	April 1972	Polychotomous Assay	Mr. Yamada
379	October 1971	April 1972	Polychotomous Assay Check	Dr. John Gurland
380	October 1971		Punch Code List - PPD Parke-Davis	Dr. O'Malley
381	October 1971	March 1972	Grass Pollen Extracts	Dr. Baer
382	November 1971		Lymphocyte Grouping with Defined Antisera	Dr. Morgan
383	November 1971	November 1971	Measles Strain Discrimination	Dr. Albrecht
384	November 1971	December 1971	Grade Progression	Mr. Ingalls
385	November 1971	November 1971	Geometric Means	Dr. Friedlaender

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TABLE I - continued

SAN	DATE INITIATED	DATE COMPLETED	TITLE	RESEARCHER
386	November 1971	February 1972	CCA, HA Relation	Dr. O'Brien
387	December 1971	December 1971	Geometric Means	Dr. Hardegree Dr. Singer
388	December 1971	December 1971	Chi-Squares	Dr. Morris
389	December 1971	February 1972	CCA Values	Dr. O'Brien
390	December 1971		T-Tests	Dr. Hardegree
391	December 1971	March 1972	Gamma Globulin Breakdown Study	Dr. Aronson
392	January 1972	February 1972	ED50 Calculations III	Dr. Hardegree
393	January 1972	February 1972	ED50 Calculations I	Dr. Manclark
394	January 1972	February 1972	ED50 Calculations II	Dr. Manclark
395	January 1972	February 1972	Plaque Size Stability	Dr. O'Brien
396	February 1972	February 1972	Flu probits	Dr. Murray
397	February 1972		Spectrometer Precision	Dr. Gerber
398	February 1972	February 1972	Geometric Means	Dr. Friedlaender
399	March 1972	March 1972	P-D 123-12	Dr. Baer
400	March 1972	March 1972	Tests of Parallelism Part I	Dr. Seligmann Dr. Baer

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TABLE I - continued

SAN	DATE INITIATED	DATE COMPLETED	TITLE	RESEARCHER
401	April 1972	April 1972	Relative Potency	Dr. Murray
402	April 1972		Tests of Parallelism Part II	Dr. Seligmann Dr. Baer
403	April 1972	April 1972	Geometric Means	Dr. Friedlaender

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Annual Report of the Office of Investigational New Drugs
Division of Biologics Standards
July 1, 1971 - June 30, 1972

This Office has the continuing responsibility for the receipt and evaluation of "Notices of Claimed Investigational Exemption" for new biological products.

During the period covered by this report 94 "Notices" were received. This brought to 608 the total number of "Notices" received since the Food and Drug Regulations requiring submissions became effective in October 1962. Disposition of "Notices" received during the period 1963-1972 is shown in the following table:

Active	330
Withdrawn	276
Forwarded to FDA for processing	2
Total	608

The number of supplements received on active INDs throughout the year depends on the activity and number of investigators involved in the respective clinical trials. Following initial review of the "Notices," this Office has the responsibility for informing sponsors of deficiencies in the submissions. Liaison with the sponsors and the Food and Drug Administration is maintained.

The information contained in these "Notices" may indicate to the scientific staff of the Division the paths in which research should be directed for the purpose of developing Regulations for monitoring the safety and efficacy of new biological products. Sponsors of INDs are encouraged to submit all available data for review so that judgments as to the safety and efficacy can be made well in advance of the time of licensing.

The members of this Office participate actively in the scientific life of the DBS and of the National Institutes of Health attending conferences and seminars as well as representing the Division in a liaison capacity with appropriate committees of other institutes. Conferences are held periodically with representatives of manufacturers and others interested in investigational new drugs.

Annual Report of the Office of Scientific Communications

Division of Biologics Standards

July 1, 1971 through June 30, 1972

The Office of Scientific Communications is responsible for establishing objectives and program policy to meet the scientific and technical information needs of the control and research programs of the Division. It carries out a scientist-to-scientist information program that provides advice and assistance and documentation on the vital scientific decision-making issues.

The office carried on its usual routine services of former years -- 6 master subject files, library activities, coordination of the Annual Report, and the processing of the technical reports generated by contracts -- with an emphasis this year on salvaging as much of DBS history as possible in the form of monographs. The first of these -- poison ivy -- was initiated at the request of Dr. Harold Baer. Monographs for polio, measles, and rabies vaccine are in the planning stage.

The informal information network for sources of information has greatly expanded with more and more collaborative work being done with the outside scientific community with mutual interests.

By far the greatest time and energy and emphasis has been devoted, during this reporting period,to the gathering and evaluation of data and information on both the components of biological products and on the products themselves. (see list below). The goal is to have all the known data and information on every aspect of every licensed biological product -- especially adverse reaction data -- in our files and readily available for decision-making at both the scientific level and top management level. A proposal has been offered to mechanize this process.

The list below highlights the areas of greatest concern &/or investigation where information was gathered or where the OSC took as active part in initiating investigation and documentation.

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1. The evaluation of the mercury content of biological products, with emphasis on merthiolate as a preservative in biological products.
2. Adverse reactions of triple vaccine use, aluminum, rabies, measles, cholera, pertussis, rubella, mumps, influenza and polio vaccines, and diphtheria antitoxin.
3. Reevaluation of smallpox vaccination.
4. Infectious hepatitis problems.
5. Hypersensitization.
6. The possible hazards of polychlorinated biphenyls contamination of biological products in the course of production.
7. Oncogenesis and adenovirus.
8. Aflatoxin.
9. 1-adamantanamine
10. The possible teratogenic and mutagenic components of biological products.
11. Aspirin toxicity
12. Ascorbic acid mechanism of action
13. Post vaccination problems
14. Aluminum granulomas and encephalopathy.
15. Adjuvant reevaluation.
16. Isoniazid toxicity and tumor formation and hepatic damage.
17. Evaluation of combined measles, rubella, mumps, and chicken-pox vaccines.
18. Immunologic methods for neoplasm diagnosis.
19. Proflavine and acriflavine
20. Evaluation of parabens tumor capacity and toxicity.
21. Evaluation of Tween 60 & 80 toxicity and tumor capacity.
22. Evaluation of bacteriocidal agents
23. Encephalopathies following prophylactic pertussis vaccine.
24. Carcinogenicity and toxicity of aluminum compounds.
25. New and novel methods for determining residual moisture in dried solids, especially detecting small amounts in small samples.
26. Phenol toxicity and adverse reactions.
27. Laboratory assays of tetanus and diphtheria toxoids.
28. Versene adverse reactions.
29. Role of histamine in tissue rejection and graft rejection.
30. Prothrombin isolation and purification.

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31. Exchange transfusion / adverse effects
32. Epidemic hemorrhagic fever prevention and control
33. Dog, duck, rabbit immunology
34. Horse and goat disease
35. Fatty acids / adverse effects
36. Herpesvirus / immunology
37. Hydrocarbons / adverse reactions
38. Delayed hypersensitivity
39. Diphtheria / complications and pathology
40. Virus diseases / veterinary
41. Chickens and virus diseases
42. Plague vaccine
43. Permeability problems
44. Bacillus anthracis / cell wall and capsule
45. Respiratory syncytial virus / immunology
46. Burkett's lymphoma / microbiology
47. Herpes viruses
48. Hepatitis associated antigen / isolation and purification
49. Blood coagulation factors
50. Pertussis vaccine / toxicity
51. Lymphocytes / cytology
52. Monkey / immunology
53. Pseudomonas aeruginosa / immunology
54. Poxvirus and oncogenic viruses
55. Ascorbic acid / metabolism
56. Infectious mononucleosis and review

A major project of the OSC to which knowledge is continually being added and evaluated is the possible hazard or toxicity or carcinogenic aspects of the chemical components used in the preparation of vaccines.

A photo-story initiated by this office, tentatively titled the history of biological products, will be used as the basis for a standing permanent exhibit in the Division. This will be a collaborative effort between the OSC and the Public Information Office.

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The strengths and weaknesses of the OSC and its struggles to serve the needs of the Division in the best interests of the public deserves mention this year.

The main strengths lie in a wide resource of information contacts -- especially in the area of toxicology, pharmacology and biochemistry. The rapport with these sources makes it possible to obtain data and information in current areas of research where publication may not even be contemplated or at least not yet generally available. The Scientific Communications Officer has a long standing working relationship with members of the larger scientific community outside the Division, many of whom are directors, presidents, or senior scientists of institutes, industries, agencies, or organizations who have knowledge highly beneficial to the DBS mission, just because they have their finger on the pulse in their specific field.

The weaknesses of this office lie in the tools -- including personnel -- to most effectively get the job done. These are universal needs across the board at DBS. We need a better understanding and recognition of what these really are and how best to resolve them. This Division has been so polarized by so many battles running simultaneously that all suffer, not just the laboratories.

Experience and history certainly say the public is best served by a multiplicity of disciplines working together -- a wide range of research and regulatory -- control -- activities working together. This is the posture this office tries to serve.

Annual Report of the Public Information Office
Division of Biologics Standards
July 1, 1971 - June 30, 1972

As a result of Dr. Anthony Morris' grievance procedure against the DBS and subsequent press coverage of Senator Ribicoff's news release of October 15 regarding Dr. Morris' allegations, the Public Information staff has been primarily occupied in providing factual information to the media and the public refuting these allegations. Nation-wide involvement with press, radio, and TV was progressively heightened with the issuance of frequent statements by Sen. Ribicoff in the Congressional Record, the release of the NIH Ad Hoc Committee to Review Morris/Turner Allegations Report, the Government Accounting Office Report, the delegation of authority to DBS re efficacy of biologics, Federal Register publication on review of bacterial vaccines with no U. S. standard of potency, and the NIH Report on Review of Managerial and Personnel Conditions in the Division of Biologics Standards.

I. PRESS: Polio vaccine in human diploid cells--news release issued July 30 on proposed standards for polio vaccine in human diploid cells (HDC). News coverage of amendment to Pfizer's license for production of polio vaccine in HDC included Hospital Practice, Consumer News, New York Times, Chem. & Eng. News, JAMA Medical News, Newsweek, Infectious Diseases, Wall Street Journal.

Monkey diploid cells--news release on collaborative contract studies leading to the development of monkey diploid cell lines as candidates for use in vaccine production was issued simultaneously Sept. 21 with presentation of the work at International Congress for Microbiological Standardization. Subsequent public criticism by Dr. Hayflick (proponent of human diploid cells) of the DBS cell line studies stimulated wide press interest, resulting in a heavy volume of press queries to this office.

Bacterial genes in human cells--a joint news release with NIMH Oct. 8 issued simultaneously with publication of the work in Nature, describing the work as "of the greatest and most startling interest to all biologists..." The story received nation-wide press coverage, as well as coverage by British, French, and German publications. Photos were also furnished press by this office.

Blood banking--Congressman Veysey's press conference (Nov. 17) proposing elimination of hepatitis by establishing an all volunteer donor program has resulted in voluminous queries and requests for background information on Federal regulation of blood banks from press and public. Press included Washington Post, Evening Star, National Observer, Chicago Daily News, Hospital Practice, Science, Drug Research Reports, Newhouse News Service, and wire services.

Purified Protein Derivative (PPD) potency--a news release Feb. 10 concerning the lack of uniformity in potency of PPD and corrective steps taken released on DBS mailing keys to selected science writers whose readers would be affected by the action. This office coordinated release through the CDC, thus ensuring orderly dissemination of information to state health officials. Coverage by medical and trade publications, included JAMA, Chem. & Eng. News, PMA Newsletter, Infectious Diseases, and Hospital Practice. A taped interview with

Dr. Seligmann, DBS, and Dr. Wismuller, CDC, on aspects of PPD production and use was arranged at the request of the American Society of Hospital Pharmacists. The ASHP distributed copies of the tape to its members (reaching approximately 5,000 hospital pharmacists) in March.

Hepatitis associated antigen (HAA) blood tests. DBS research on HAA tests for transfusion blood were covered by science writers in Medical World News, Infectious Diseases, Patient Care, Clinical Laboratory Forum, Internal Medicine News, and Hospital Practice. News release issued in May '72 at time of publication in Federal Register requiring that all transfusion blood be tested to eliminate blood positive for HAA.

Additional items of press interest: Purification of influenza vaccine components reducing the potential for causing adverse side effects, elimination of older strains from vaccine components, and the increase of active virus material per dose; licensing of additional manufacturers (now total 9) of hepatitis associated antibody (press release issued Feb. 19, '71 at time of first license); illegal interstate sale of pregnancy test kits for home use; so-called "Hadley cancer vaccine."

II. RADIO AND TV: CBS (TV) News blast on flu and adeno vaccines: Following a request (Oct. 4) by CBS for filmed interview with DBS Director on CDC immunization programs (measles, polio), the interview (Oct. 5) was unexpectedly slanted toward discussion of flu and adeno vaccines, with emphasis on questions raised in Dr. Morris' grievance procedure against DBS.

NBC Chronolog, Oct. 22, on blood banking: Tom Pettit, NBC producer, requested access to DBS files on licensed blood banks. Request was submitted under Freedom of Information Act. This office coordinated requests and release of information thru DHEW Information Center Officer. Activities of various national organizations concerned with blood banking, as well as DBS, were covered in the documentary.

WGMS "Discussion: NIH"--Two interviews on cholera (Nov. 15 and Feb. 18) with Dr. Robert Northrop by Win Clearwater.

III. SPECIAL PROJECTS: Fact Sheets: 1) "Facts about Donor Blood for Transfusion," 2) "Bacterial Vaccines with No U.S. Standard of Potency," and 3) "Problems with Influenza Vaccine"--prepared for use in answering queries from press and public.

Seminar: for 20 visiting French physicians Oct. 7 arranged in cooperation with ODI and FIC. Topics included "Viral Diseases and Vaccines," "Cancer Research," and "Cardiovascular Disease," with speakers from DBS, NIC and NHLI.

Chapter on blood banks for 1972 edition of Health Resources Statistics--assisted National Center for Health Statistics staff writer in preparation and clearance of chapter.

Review article on DBS--furnished extensive background material for Ron Maselka's article (April 26), Buffalo Evening News.

Awards--1971 Kennedy International Awards to Drs. Meyer and Parkman--furnished biographical material and photos of Drs. Meyer and Parkman, as well as film clips depicting their research for use at awards ceremony.

IV. DISTRIBUTION OF DBS PUBLICATIONS: Literature distributed on request included DBS Brochure; PHS Advisory Committee on Immunization Practices Recommendations; Blood and the Rh Factor; Rubella Fact Sheet; DBS Highlights of Research; and follow-up requests for reports listed in introduction.

V. RECURRING PROJECTS: NIH Directory-Bibliography; DBS 1971 published papers bound; NIH Almnac revision; NIH Record stories on staff awards, appointments, retirements; etc.; NIH Calendar announcements and distribution of fliers for DBS research seminars; DBS Highlights of Research (for Congress); DHEW Annual Report; NIH Annual Report of International Activities (DBS international activities); NIH Publications List (semi-annual revisions); DBS Who's Who, 1972 revised for staff use only; NIH Annual Report.

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